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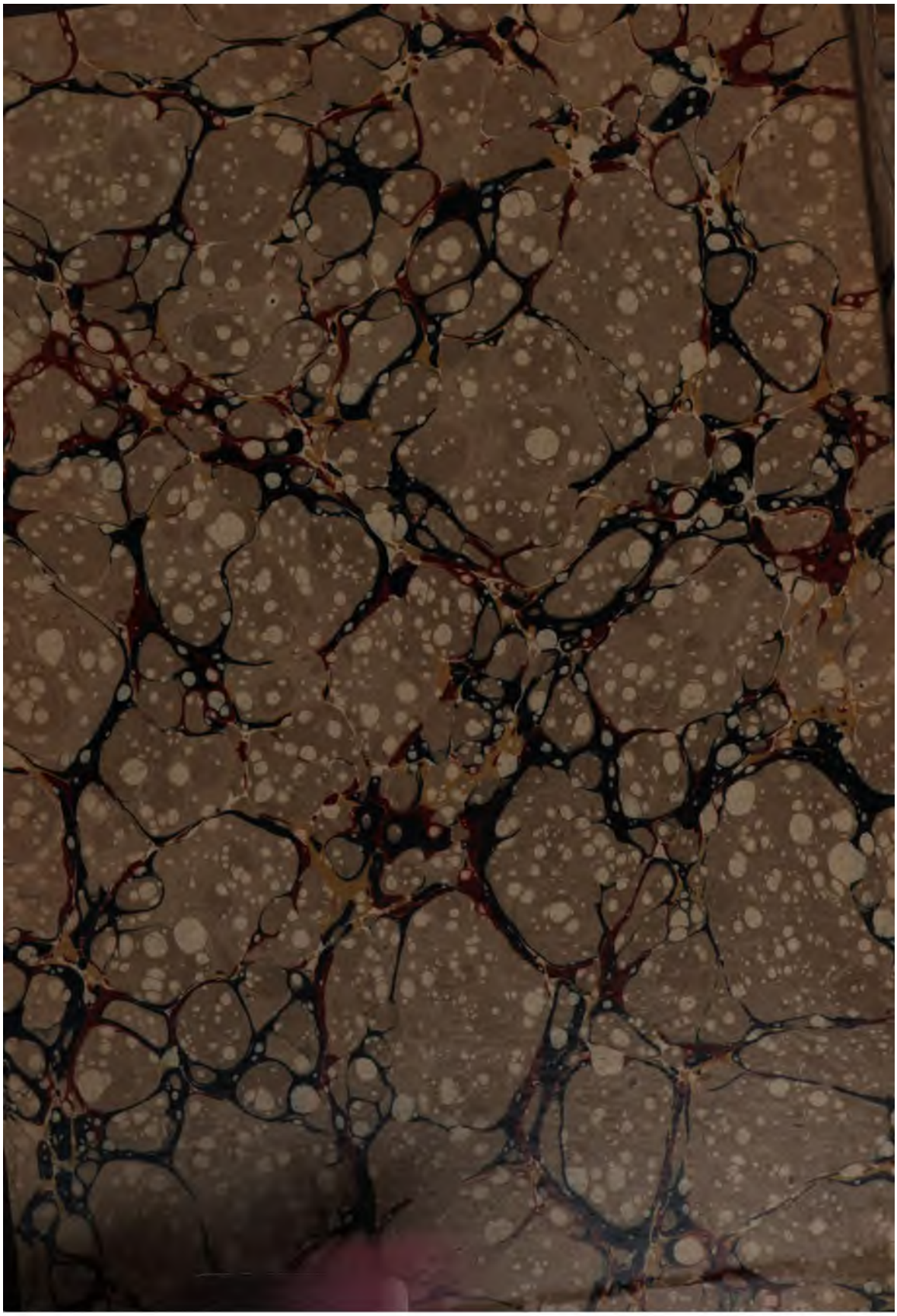

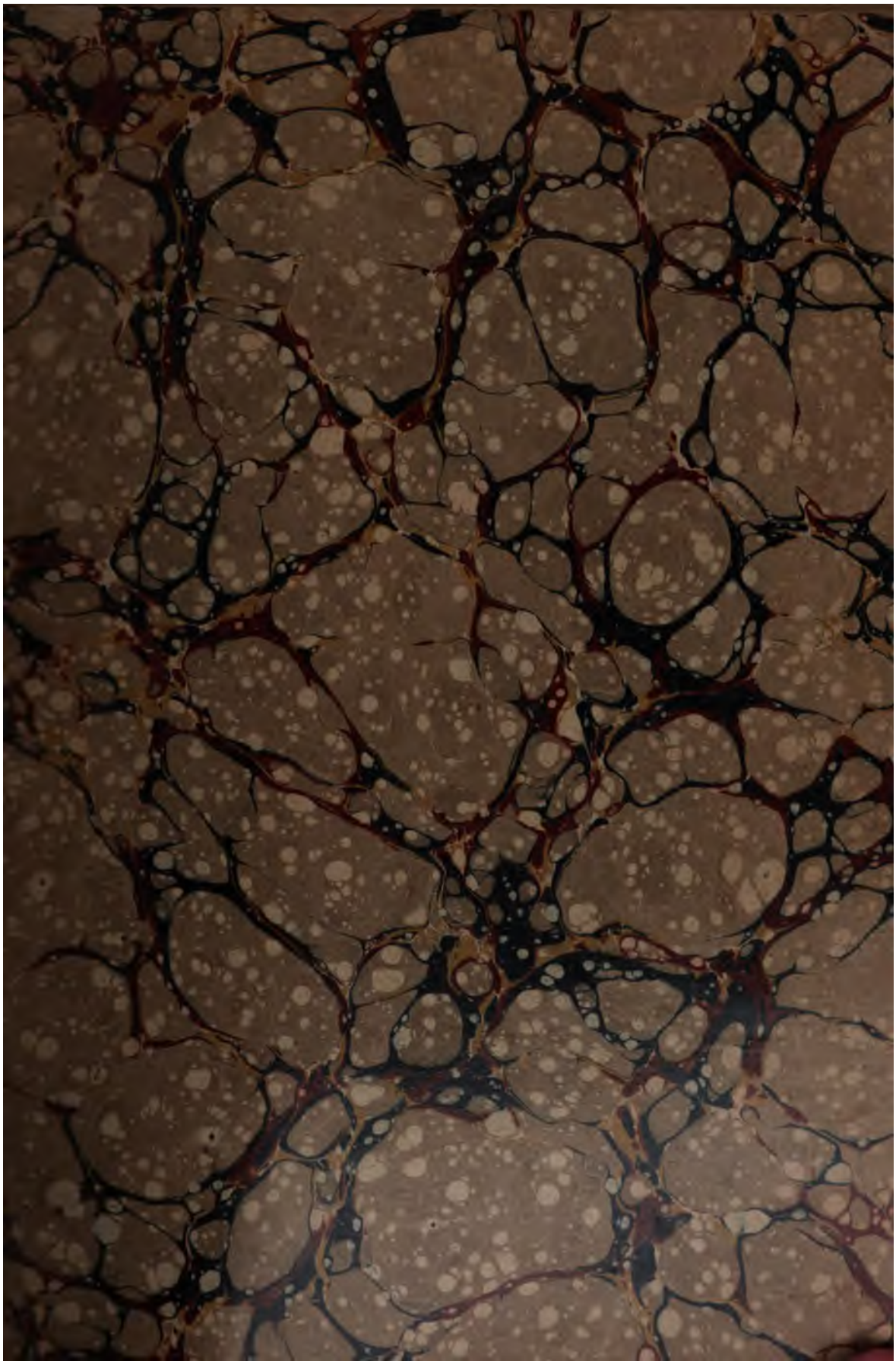

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XIV DECEMBER, 1907 NO. 1

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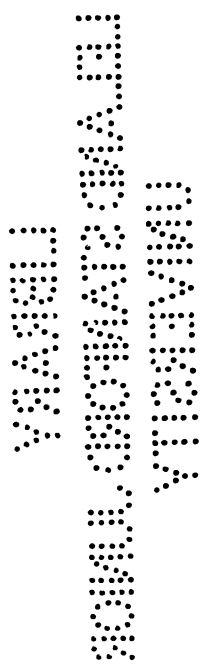

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BIOLOGICAL BULLETIN

ON THE LIGHT RECEPTIVE FUNCTION OF THE MARGINAL PAPILLÆ OF GONIONEMUS.[1]

L. MURBACH.

INTRODUCTORY.

On the oral or subumbrellar margin of the jellyfish *Gonionemus*[2] there are somewhat spherical or cordate papillæ, corresponding in number to the tentacles, and almost directly underneath the proximal ends of the tentacles. They are a bright translucent rust color with darker pigment lining the interior. The cavity is a continuation or pouch from the circular canal, thus insuring abundant nourishment for them. To distinguish them from marginal bodies, by which name Cœlenterate writers generally designate the otocysts, these organs are called marginal papillæ. Nutting[3] calls them sense bulbs, and Hargitt[4] and Goto[5] refer to them as "basal bulbs," Hargitt[4] describing them as "of brownish color delicately tinged with bright green." He also ascribes visual function to them.[6] The beautiful green spots

[1] To the management of the Marine Biological Laboratory I owe the material assistance enabling me to carry on my work and for this I am much indebted.

[2] The Woods Hole species is, of course, the one in question. Hargitt in "The Medusæ of the Woods Hole Region," p. 53, under the synopsis of *Gonionemus murbachii* Mayer, says: "This species was first described by A. Agassiz in 1862 from the Pacific coast. In 1895 a species was found at Woods Hole and supposedly identified with the Pacific species by Murbach, but it has since been classified as a distinct species by Mayer." This "supposition" is gratuitous. By using only the genus name in my report I meant to indicate my doubt about the identity of the species.

[3] Nutting, C. C. ('01), "The Hydroids of the Woods Hole Region," U. S. Fish Commission Bulletin, 1899 (Publ. 1901).

[4] Hargitt, Charles W. ('04), "The Medusæ of the Woods Hole Region," Bulletin of the Bureau of Fisheries, Vol. XXIV., 1904.

[5] Goto, Seitaro ('03), "The Craspedote Medusa *Olindias* and Some of its Natural Allies," The Mark Anniversary Volume, Art. I., pp. 1–22, 1903.

[6] *Loc. cit.*, introduction.

are in the bases of the tentacles, due apparently to refraction and reflection from brown pigment granules in the free ends of the endoderm cells of the tentacle insertion. As I understand that "basal bulbs" refers to the enlarged proximal ends of tentacles, or bulbs into which the tentacles are inserted, I do not use this expression.

To these marginal papillæ, under the name of marginal bodies, Yerkes [1] ascribes a special light-recipient function; he says, "the probability is strong that they are the special organs of photic stimulation." This conclusion seems to be based on the location and heavy pigmentation of the papillæ, and also on some experiments which will be referred to farther on.

In the Challenger Report on the Deep Sea Medusæ (Vol. IV., p. lii), Haeckel said: "As experiment showed, it is principally the swollen bases of the tentacles which bear such pigment eyes, and that chiefly in the order of the Anthomedusæ and in those Leptomedusæ which have no marginal vesicles. Such ocelli are more rarely found in Trachomedusæ, Narcomedusæ, and Stauromedusæ." Again he says: "Moreover medusæ perfectly devoid of color which have neither marginal ocelli nor other pigment spots, are sensible to light; in this case it is probably the sense epithelium of the umbrella margin which discharges this function."

Now wherever there are special structures in the bulbs above referred to by Haeckel there is perhaps, no doubt that they serve for receiving stimuli of some external kind. In the Leptomedusæ and Anthomedusæ there are true tentacular bulbs and the pigment spots in them may serve in the reception of light. On the other hand, in *Gonionemus*, a trachomedusa,[2] the marginal papillæ are not tentacle bulbs and have apparently no special structures for light reception, unless we consider the presence of

[1] Yerkes, Robert M., Ayer, James B., Jr. ('03), "A Study of the Reactions and Reaction Time of the Medusa, *Gonionema murbachii*, to Photic Stimuli," *American Jour. Physiology*, Vol. IX., No. 5, 1903.

[2] While there can be little doubt about some of the positive trachomedusan characters of the jellyfish, Dr. A. G. Mayer has suggested to me that in the light of Goto's careful work (*loc. cit.*) classing it as a leptomedusa renewed examination of the otocyst organs would seem desirable. As this subject merits more space than can here be given I will only say that I have prepared new sections and carefully examined a rather large series and am convinced that the otocyst organs are derived from the endoderm.

much pigment to answer this requirement. In regard to this point Beer[1] ('01) said: "Mit dar viel zu alt gewordenen Ansicht, dass Pigment der wesentlichste und ursprüglichste Bestandtheil eines jeden Sehorganes sei muss endgültig gebrochen werden." And in a considerable paper he makes good this statement.

In regard to the structure of the marginal papillæ it may be said that most Trachomedusæ and some Leptomedusæ possess a welt or ring of tissue made up of ectoderm, enlarged especially at, or near, the bases of tentacles by the rapid growth of cells for the production of nettling organs[2] which are to migrate out on the tentacles. In Goto's description of *Gonionemus depressum*[3] it is stated that "these bulbs . . . contain hollow prolongations of the entoderm of the circular canal, and the ectoderm is clogged with developing nettle cells." Perkins shows this structure in two figures.[4] The main point is that there is here no definite arrangement of either cells or pigment such as is present in so-called primitive eyes or eyespots. So that these papillæ would scarcely be expected to function as special organs for light stimulation. This does not preclude their being tactile; they are ciliated.

From experiments Yerkes finds[5] that when the oral surface is up, *Gonionemus* is much more sensitive to light than when the aboral surface of the animal is turned toward the light. This is supported by his observations that in a weak light the bell margin is turned upward and in strong light it is turned downward. The tentacles are cut off and, testing the animal, he finds that while the tentacles are sensitive to light they are not the cause of the difference in sensitiveness between the oral and aboral surfaces of the medusa. After removing the whole margin of the bell Yerkes finds that the remainder of the bell is not sensitive to light, or at least does not provoke response. Then

[1] Beer, Theodore ('01), "Ueber primitive Sehorgane," 1901.

[2] Murbach, L. ('94), "Beiträge zur Anatomie und Entwicklung der Nesselorgane d. Hydroiden," *Archiv für Naturgeschichte*, 1894.

[3] *Loc. cit.*

[4] Perkins, Henry F. ('02), "The Development of *Gonionema murbachii*," *Proceedings of the Acad. of Nat. Sciences of Philadelphia*, November, 1902 (March, 1903).

[5] *Loc. cit.*, page 300.

he adds (p. 302) that animals, whose tentacles were removed in such a way as to destroy the marginal bodies, never reacted to light; this, casually mentioned, as if it were not important. Yet this should be the crucial experiment.

Now since the marginal papillæ in *Gonionemus* are not true tentacle bulbs, and are only connected with the tentacles by the outer layer of ectoderm, and since the tentacles when pulled off by merely seizing them always, in my experience, break off at their bases, leaving the latter, I could not see how the operation above referred to could be performed. It would be possible by seizing papilla and tentacle-base with forceps, but this would remove the margin of the bell with the nerve ring.

Problem.

These considerations led me to make experiments, first: to determine whether the light reactions of *Gonionemus* are dependent on stimulation of the marginal papillæ, or, second: whether any other set of organs, *e. g.*, the tentacles, the velum, or the gonads, also have this function.

Methods.

The first experiments were made by cutting the papillæ away from the margin with fine sharp scissors in such a way as to injure as little as possible the adjacent parts. Operated animals were left until they had recovered from the shock, or even for several days, but never long enough to allow the papillæ to regenerate. They were always compared with normal animals taken at the same time. Colors were also noted, as was the temperature of the water. In some of the experiments a 5 cm. deep saturated alum solution was interposed between the animals and the sun, but I have found with other experimenters, that this gives no advantage over plain water. The temperature varied no more than from one fourth to one and one half degrees centigrade in any of the experiments during an observation, and since the only purpose was to test the mere reaction of the papillæ or other organs, this change in temperature could be ignored. The medusæ were exposed in dishes of white glazed earthenware, in glass dishes, and in dishes with black substratum. It may

be remarked here that in the latter case the reactions were generally slower. The exposure was made in sunlight varying as it does during the middle portion of the day. For shutting out the sun absolutely opaque material was used; in some cases plain covers, in others covers that would completely darken the dish. While these give varying results in reaction time, they will not be considered here since constant conditions were maintained throughout any one experiment. In accurate time reaction experiments there should be some means of determining the exact intensity of light used. In some cases the exposure was uniformly for 60 seconds; in the earlier experiments only until reaction took place. The rest period I varied from 30 to 300 seconds; in general I found the former too short and the latter too long. Contraction leading to displacement was counted a reaction. From five to ten observations were made in succession and tabulated, but space will not here be taken for the tables in full. Only about one half the averages obtained will be given. It will be seen that I have followed somewhat generally the methods of Romanes,[1] Conant and Berger,[2] and Yerkes.[3]

Having received response to light in all cases where the marginal papillæ had been carefully removed, another cause of the reaction was looked for. Now as there is a thin welt of tissue connecting the papillæ which appears to be made up of similar cells, having about the same color, this welt of tissue might be conceived as taking over any light-percipient function that the removed papillæ were thought to possess; it was accordingly also removed. As this is a much more painstaking operation the work necessarily progressed slowly. In some cases a method was employed somewhat similar to the one previously adopted in removing the otocyst organs in the same medusa.[4] In other cases I depended on cutting away as little of the margin as possible and yet removing the welt of tissue in question, together with the papillæ. Such operated medusæ were usually tried in

[1] Romanes, G. J. ('76), "Jelly-fish, Star-fish and Sea-urchin," International Sc. Series, 1885.

[2] Berger, E. W., *Mem. Biol. Lab. Johns Hop. Univ.*, Vol. IV., 4.

[3] *Loc. cit.*

[4] Murbach, L. ('03), "The Static Function in *Gonionemus*," *Am. Jour. Physiol.*, Vol. X., No. 4, December, 1903.

from one or two to three days after the tissues in question were removed.

Results.

In all cases where papillæ were removed reactions still continued. As soon as such animals as had also the nettle welt removed were tested, the experiments were seen to be confirmatory of those on the papillæ. There was always a definite response to light stimulus, but in some cases it was slow. And it is still noticeable that the oral side of the medusa was more sensitive to light than the aboral. It ought to be stated here that the velum was always removed with the welt of tissue and it was not necessary to wait for its regeneration in order to get responses to light.

To determine if any of the other marginal organs or even any of the organs on the oral side of the animal had anything to do with the light perception, exclusively, or whether, as I began to think, it was the function of more than one organ — perhaps the subumbrellar epithelium in general — other organs which it was thought might be stimulated by light were removed. The ovaries, the velum, and the tentacles were in turn cut away. It is difficult to remove the velum entirely, as the closest cutting that can be done with the finest scissors leaves a narrow bit of the attachment of the velum on the margin. However, since the velum may be entirely removed while removing the welt and papillæ, a differential result will give approximately the value of the velum. Although these operations are more or less severe for the size of the organism, yet I have never observed so severe a shock from any of them as recorded by Yerkes, viz., that they have not again recovered. Indeed, I have almost always removed all the tentacles together and do not remember to have lost any individuals from this cause.

A preliminary experiment on a normal one, one without ovaries, one without tentacles, one without the welt and papillæ, resulted as follows; For the normal the average reaction time was $7\frac{1}{6}$ seconds; for the one without ovaries $10\frac{3}{8}$ seconds; for the one without tentacles $20\frac{5}{7}$ seconds; for the one without nettle welt and papillæ $12\frac{1}{5}$ seconds. In the light of later observations these results, except the last, seemed too long, as those without ten-

tacles or gonads displayed a remarkable activity, probably due to the removal of these organs.

In one of the first experiments a normal medusa was compared with two of the same lot in which the marginal nettle welt and papillæ were removed. The averages of ten trials were as follows: Average reaction time of the normal medusa $8\frac{3}{5}$ seconds; a 12 mm. olive-colored operated medusa $9\frac{1}{2}$ seconds; a 6 mm. yellow orange operated medusa 12 seconds. Next to this in reaction time were the medusæ with gonads removed. The average of four sets of experiments was $13\frac{1}{3}$ seconds. For the velum the average was higher, being $27\frac{2}{7}$ seconds; and for the tentacles still higher being 29 seconds. Another case, four hours after removal of the tentacles gave an average of four seconds.

Discussion.

Now, comparing these, account must be taken of the operations necessary for the experiments. The removal of the tentacles is the simplest of all and least likely to prejudice the action of other parts, therefore these results are the least doubtful. The careful removal of the gonads can have very little detrimental effect, as no principal part of the nervous system is involved. The removal of the marginal papillæ may include some of the underlying tissues, and more of this is included when the nettle welt is removed in addition. The same is true when the velum is completely removed. In the experiment for the value of the velum the attaching margin had to be left in order not to injure the nerve ring too seriously. In view of these facts negative results after any similar operation, such as Yerkes obtained for the removed papillæ, must be looked upon as needing confirmation in other ways, rather than being considered a proof of the function ascribed to the organs under consideration. In the specimens without the attachment of the velum, and those with the nettle welt and papillæ removed we must bear in mind that parts of the nervous system are more or less affected.

Conclusions.

From the earlier experiments of removing the papillæ alone it is evident that they are not exclusively the organs of light stim-

ulation. From the later experiments we may conclude that the welt of pigmented tissue running around the margin of the bell from papilla to papilla is not important, if at all sensitive to light. And furthermore that the ovaries, tentacle and velum have practically no more to do with this function than other organs. Injury to the nervous system may account for the slow reaction when the margins were removed for marginal welt of tissue and the velum. If now none of these organs that have been tested are solely affected by light, indeed only seem to slow the reaction in proportion to the injury, it seems to indicate that the epithelial tissue on the subumbrellar surface, in general, is the responsive organ.

PRELIMINARY NOTE ON THE DISTRIBUTION OF THE TIGER BEETLES (CICINDELA) AND ITS RELATION TO PLANT SUCCESSION.

V. E. SHELFORD.

The adult beetles are graceful, predatory, swift-flying insects whose definite distribution and great variability have long been matters of comment. The larvæ have been found to be more circumscribed and definite in their distribution than the adults. Our attention has accordingly been turned to the behavior of the larvæ and of the adults at the time of egg laying for a possible explanation.

The egg laying habits are simple. The last four segments of the abdomen are used as an ovipositor. Two pairs of the appendages of these segments serve as digging organs with which small vertical, well-like holes from 7 to 10 mm. deep are made in the soil. A single egg is deposited in a hole and the hole is left uncovered. The tenth segment of the abdomen and one pair of appendages of the ninth segment are covered with hairs which are probably associated with organs sensitive to the varying degrees of soil moisture and the size of soil particles. The females try the soil before depositing eggs. They make many holes, but lay in only a part of them, and frequently discard them before the usual depth is attained.

The larvæ almost always remain in the spot where the eggs were laid. Upon hatching each larva constructs a burrow in the place of the ovipositor-hole and reconstructs and enlarges the burrow after each moult. If a larva migrates, it almost always selects the same kind of place for digging a new hole as that in which the eggs were laid.

So much for the general aspects of the life habits. Let us now turn to some examples of distribution and behavior during the egg-laying time.

The larvæ of *Cicindela purpurea limbalis* are found on steep

clay banks.[1] The range of the adults is far wider. To determine the cause of this distribution, adults were placed in cages containing soil of several kinds. Each kind was so arranged into steep and level parts that about one square foot of each type was exposed. The adults placed in the cages were taken when the species was copulating freely.

The following table shows the number of larvæ which appeared in the case of three lots of *C. purpurea limbalis.*

Soil.	Humus.		Clay and Humus.		Clay.		lean Sand.		Sand and Humus.	
S = Steep, *L* = Level.	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*	*S*	*L*
I. No. of Larvæ.	0	0	0	0	9	0	0	0	0	0
II. No. of Larvæ.	0	0	0	0	12	1	0	0	0	0
III. No. of Larvæ.	0	0	1	0	24	10	0	0	0	0

Other pairs taken in coitus were placed in cages containing sand only and level clay only. No larvæ appeared in either case. Females placed in cages containing rough steep clay, deposited eggs. Similar experiments have been carried out on several other species and it becomes apparent, therefrom, that the local distribution is determined by the egg-laying instincts. Since the animals cannot and do not continuously remain far from the breeding place, the breeding place becomes the true index of their habitat. Their local distribution being determined by egg-laying instincts, in other words by the life needs, and housekeeping habits of the animals, it may be called "ecological distribution" from Haeckel's[2] definition of that term, and the etymology of the word.

Habitat selection in correlation with geological factors such as erosion and deposition, and with the succession of plant formations and societies forms one of the great factors of dispersal, isolation, etc.

[1] The nomenclature used in this paper is to be found in Horn's "Systematischer Index der Cicindeliden," *Deut. Ent. Zeit.*, Feb., 1905, Supplement. *C. scutellaris* Say, however, stands in that publication as *obscura* Say aber. *Leconlei* Hald., the corresponding change having been made by the same author in a later publication.

[2] Ecology is the science of the domestic side of organic life, of the life needs of organisms and their relation to other organisms with which they live. "Wonders of Life," 1905.

The relation of the distribution of *Cicindela* to the succession of plant societies has been especially studied in the vicinity of Chicago. The area which affords the basis of this study is to be found at the south end of Lake Michigan. Conditions here since glacial times have led to the deposition of large areas of sand, which in the eastern portion of the field of deposit, is stretched over an area of several miles wide. At the point where most of the studies have been made there is a series of ridges which were originally thrown up under water and later added to by aerial deposition. These ridges are separated by long depressions, most of which contain water. The structures are accordingly arranged in a horizontal series, the oldest being, of course, furthest from the lake, and differing from the younger only in age, and in being a little less exposed.

A definite succession of plant societies has been worked out by the plant ecologists (Cowles and Clements) and this succession is due largely to the conditions necessary for the germination of the seeds and growth of the seedlings of the different plants. In forest development, before the climax stage is reached, the seeds of the trees comprising a given stage do not germinate and their seedlings do not develop in the shade of the forest then present. Each stage accordingly prepares for another and more mesophytic type. The trees of the climax stage of eastern North America, the beech and the maple, produce seeds that will germinate in that forest's own shade. Accordingly the beech and maple will last indefinitely.

Not all of the conditions herein described occur in the horizontal series at any one point, but all are to be found within sandy areas near Chicago.

Let us start with the strip next to the water's edge, the very youngest deposit. It is frequented by the adults of *C. cuprascens* and *hirticollis*. The larvæ of the latter are sometimes found here, but more frequently a little further back on the low, wet places on the beach. Other ridges are seen to be formed beneath the water and this margin is accordingly potentially the first depression.

On the lakeward side of the first ridge, among the young cottonwoods, we find the larvæ of *lepida*, the white tiger beetle. On the leeward side where bunch-grass has come in and the cotton-

woods are old with occasional seedlings of gray pine intermixed, we find the larvæ of *C. lepida* displaced by those of *C. formosa* var. *generosa*, which reach their dominance among the young pines.

Coming in on the ridges with the pine are the larvæ of *C. scutellaris*. In our horizontal series this species is to be found further from the lake than any others yet mentioned. As new ridges are thrown up outside of a given one and as it becomes older, the differences between the lakeward and the landward exposure quickly disappear.

Let us turn our attention for a moment to depressions. We have noted that *C. hirticollis* occupies a station on the white sand of the beach. In addition to occurring occasionally in the wet situations just mentioned, the larvæ are found in any of the fresh natural depressions that are deep enough to be continually moist at, or near the bottom. Such depressions sometimes occur on our lake shore, behind a first line of small dunes. As a depression becomes older and the sand becomes somewhat darkened by the decay of the reed, *Juncus balticus*, the larvæ of *C. hirticollis* give way to those of *C. repanda* which occur a little higher up than the former, on the sloping sides of the depressions. As the *Juncus* becomes thicker and a few other plants come in, the larvæ of *C. repanda* become a little less numerous. We have been able to follow this process in an artificial depression. Finally the vegetation becomes so dense as to drive out the larvæ of *C. repanda* entirely. They are succeeded by the larvæ of *C. tranquebarica* which occur still higher up the side of the depression. This stage is coincident with the development of young gray pines on the ridges.

Shrubs of various sorts appear on the depression margins at this stage and gradually increase in numbers. The first are the willows and the shrubby cinquefoils. These are succeeded by the button bush and swamp white oaks which make the depression margin too shaded for the larvæ of *C. tranquebarica* and the tiger beetle succession of the depression margins is at an end. This stage of the depressions corresponds to the establishment of the black oak, which succeeds the pine, on the ridges.

Returning to *C. scutellaris* at this stage, we find it still, in the

open places of the black oak ridges. These oaks are destined not to remain and are crowded out by the coming in of the white oaks. For an immense period after this, the habitats of *C. scutellaris* become more and more narrowed. Long before the next tree, the red oak, makes its appearance, *C. scutellaris* has been crowded out. Many centuries must pass between the coming in of the white oak and the establishment of the red during much of which time the *Cicindelas* are entirely absent. With the establishment of the red oak, conditions are ready for the next tree, the shag-bark hickory, and with it comes *C. sexguttata.* This species appears to reach its dominance in the early stages of the white oak-red oak-hickory forest, and to be crowded into its margins with the development of further mesophytism. It continues in the roads, clearings, fired places, and paths of cyclones in this forest for a long period. Individuals are sometimes to be found in the dense parts about a fallen tree. This type of forest is, however, destined to disappear and its disappearance is heralded by the coming in of the seedlings of the beech and the maple. With their appearance *C. sexguttata* becomes rare in the forest proper.

This species does not deposit eggs in pure humus, but makes use of little irregularities in clay or sand, which, contains a little humus and which is shaded slightly, such conditions as are afforded by falling trees and the erosion of hill sides by small brooks. It prefers a few loose leaves and will lay eggs under them in preference to other places when they are present. It does not, however, appear to like very shady conditions. Several days spent in the beech and maple forest has failed to reveal the presence of one of these insects although they were present in open and partially cleared places a short distance away where the forest has not become so mesophytic.

The beech and maple forest is very shady and has a floor of decaying leaves about one inch deep and several inches of very mouldy humus below these, so that there is no place in the forest proper where *C. sexguttata* can deposit eggs. It is driven out by the development of these conditions.

The white oak-red oak-hickory forest is now distributed over much of the eastern half of northern North America, but the climate in which the beech and maple will develop extends west-

ward only to the Mississippi and Illinois rivers. Dr. Cowles, whose work is still unpublished, has studied the forest of the eastern United States and has come to the conclusion that with the base leveling of the eastern plateau the beech and maple forest would, man eliminated, succeed the less mesophytic types and come to completely cover the territory extending to the western limit of its climatic range. This forest would then come to occupy the entire territory east of the Mississippi and Illinois rivers. This means the driving out of *C. sexguttata* which is now abundant in the forest of this region. Not at once, to be sure, but irregularly and gradually, first giving irregular and finally discontinuous distribution with a constant narrowing of the range of the isolated habitats. For immense ages habitats would no doubt continue to exist, but since the differences between the different elevations and brook and river margins on the one hand and the climax forest on the other become less and less as the development of the climax stage proceeds, the chances for the maintenance of habitats of *C. sexguttata* indefinitely, seem small.

The general effect of the development of these conditions on the distribution, would be as follows: *C. sexguttata* would be left only in that portion of its present range, west of the climatic conditions suitable for the development of the beech and maple forest, with possible remnants in the eastern plateau which might by a process of isolation, be caused to take on new habitats and new characters.

The general principles here set forth apply to the *Cicindelas* associated with the development of rivers and the erosion of uplands. Observations now under way go to show that they apply to the fauna in general. Strikingly different faunas are to be found in the different forest stages herein mentioned. Plant succession is then a factor which we cannot afford to neglect in considering distribution and evolution.

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ARTIFICIAL PARTHENOGENESIS IN THE SILKWORM.

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I may be pardoned, because of the brevity of this paper, for recalling attention to a subject that seems (but is not) pretty well worn. Really only three men (Tichomiroff, Verson and Quajat) have contributed the data of observation and experiment which have furnished the literature of parthenogenesis with such a host of fleeting references that it must seem to the casual reader as if silkworm parthenogenesis had been investigated only less than that of the sea-urchins. As a matter of fact it has been investigated (the work described in the present notes included) but little.

In a clutch of unfertilized eggs oviposited by a virgin silkworm moth (*Bombyx mori*) almost always a small number of eggs begins development. This development extends to the formation of the embryonic envelops and sometimes farther, and is clearly indicated to the observer by the change in color of the egg from yellow to cherry or through cherry to gray. Non-developing eggs remain yellow and, after a while, collapse. Eggs which begin to develop either persist in spherical shape, which indicates persisting life, or collapse, which means death. The development of unfertilized eggs rarely proceeds, without artificial stimulus, beyond a very early embryonic stage. In fully 500 clutches or broods of unfertilized eggs (from confined females from isolated cocoons) under observation, not a single egg gave up its larva, although an average of about seven or eight per centum of the eggs began to develop.

Although this parthenogenetic development always ceases and the embryo dies before reaching hatching stage, much difference in vitality or duration of life of the egg (strictly, embryo) is noticeable. Some of the developing eggs collapse within a few days, some in a few weeks, while a few persist for several months. (The normal egg stage, *i. e.*, time from egg laying to hatching of larvæ in the silkworm univoltin races, is about nine months.)

There is also to be noted a difference among races in the proportion of unfertilized eggs which begin to develop. Among a dozen races in our rearing rooms, one (a vigorous white-cocoon race called Bagdad) is strongly inclined to normal parthenogenesis, from twenty-five to seventy-five per centum, even in a few cases ninety-five per centum, of the eggs in unfertilized lots beginning to develop. The more usual proportion, however, *i. e.*, that shown by the other races, is, as already noted, less than ten per centum. So much for normal parthenogenesis in the species.

In 1885 Tichomiroff discovered that by bathing the unfertilized eggs with concentrated sulphuric acid, or by rubbing them gently, he could induce a considerably larger per centum than the normal to begin development. He repeated his experiments, confirming and extending his results, in 1902. By histologic examination of the eggs he learned that the artificially stimulated eggs which develop do so in a somewhat abnormal manner. Tichomiroff held the stimulus to development to be neither the action of specific ions, osmotic pressure nor catalysis. He believes that the eggs respond by segmentation to any appropriate excitation, "whatever the nature of this excitation."

Verson, in 1899, used electricity as a stimulus, and found that the development thus initiated ceased at a point about corresponding with that reached by a fertilized egg on the third day after oviposition.

Quajat (1905) submitted unfertilized eggs to the action of oxygen, high temperatures, sulphuric acid, hydrochloric acid, carbon dioxide, and electricity. His account of the experiments indicates that he was able to stimulate development by several of these agents, but he gives no data to show the proportion of developing eggs in the various treated lots. No larva issued, but by an examination of the eggs he found that several embryos had practically completed their development and growth.

My own experiments include the treatment of something over a hundred lots of unfertilized eggs (a "lot" is all the eggs laid by a single female, averaging from 100 to 350 in number), and of several lots of fertilized eggs (to serve as controls to indicate possible injury to the eggs from the reagents used). The stimuli or agents used were dry air (obtained by drawing air through vessels

of calcium chloride and then of concentrated sulphuric acid), high temperature, sunlight, friction, sulphuric acid, hydrochloric acid, glacial phosphoric acid, glacial acetic acid, absolute alcohol, potassium hydroxide, ammonia, and lime water. The reagents were used in different dilutions and for varying lengths of time. The treatment was applied to eggs not more than twelve hours old; mostly to eggs but a few minutes to a few hours old. Five hundred or more lots of untreated, unfertilized eggs were observed in order to determine the extent of normal parthenogenetic development. The eggs of half a dozen silkworm races were used and all the eggs were preserved from time of laying until their death.

As it seemed to me that most of the favorable results obtained by Tichomiroff and Quajat were obtained by treatments which had as common effect a dehydration (such as high temperature, friction, sulphuric acid, etc.) I attempted to test this first by using various dehydrating agents, especially a dry chamber in which the eggs could be submitted for from a minute or two to several hours to a nearly perfectly dry atmosphere. Friction, heat, sulphuric acid, phosphoric pentoxide and glacial phosphoric acid were also used as dehydrating agents. At the same time other treatment, not dehydrating, was used on other lots and gave results hardly less favorable than the dehydrating. The results at the end of this first course of treatment seemed to point to the hydrogen ions as the most likely development-inciting factor. Hence various agents agreeing in containing hydrogen ions though differing radically in other particulars were used. The results gave no encouragement to the hydrogen ion theory. In fact I have not been able to come to an opinion concerning the true *causa efficiens* in the matter. My results simply show to me that various stimuli, acid or alkaline, dehydrating or non-dehydrating, possessing or not possessing hydrogen ions, are able to increase materially the proportion of eggs that develop in lots of unfertilized eggs. The following paragraphs give baldly a summary of the results obtained.

Treatment of Unfertilized Eggs by Dry Air.— Freshly deposited eggs placed in dry chamber for from 14 minutes to 2 hours. Ten lots of unfertilized eggs. In all these lots, except one, a

proportion not exceeding the normal reached the gray stage. In several lots the proportion reaching the cherry (earlier) stage was distinctly above the normal. In one lot two thirds of the eggs reached the gray stage (probably a lot of Bagdad race eggs). One fertilized lot was treated to see if the drying had any injurious effect. Submitted to the dry air for thirty minutes this lot developed normally and all but ten or twelve eggs (a normal number) hatched.

Treatment with Sunlight. — Two lots of unfertilized eggs put in direct sunlight for one and two hours respectively (temperature 35° C.). Ten eggs in each lot reached gray stage, a normal number.

Treatment by Friction. — Several lots rubbed with tooth brush, not very hard. A small increase over normal average of gray eggs, some of these grays persisting alive for nine months, *i. e.*, time for hatching, but none hatched.

Treatment by Heat. — Lots heated in oven to various temperatures from 25° to 57° C. The higher temperatures caused death of all eggs, as well as eggs of fertilized lots used as checks. No increase, over normal average, of developing eggs, through use of the non-fatal temperatures.

Treatment by Phosphoric Pentoxide and Glacial Prosphoric Acid. — Nine lots treated for from one half minute to one hour, the acid applied in some cases as powder, in others as liquid solution. The records are of sufficient interest to give in detail.

Lot 1 : Treatment one hour. Acid put on as powder. Lot of sixty eggs. Treated June 6 (1906).

June 11, three gray eggs.

June 21, six gray eggs.

August 28, twenty-one or more cherry and gray eggs, of which three are alive, others dead.

Lot 2 : Treatment, one hour. Acid put on as powder. Lot of one hundred eggs. Treated June 6 (1906).

June 11, seven gray eggs.

June 21, seven gray eggs.

August 28, thirty cherry and gray eggs (one half are gray), but all are dead.

Lot 3 : Treatment, one hour. Acid put on as powder. Lot of seventy-five eggs. Treated June 6 (1906).

June 11, one gray egg.

June 21, one gray egg.

August 28, twenty-five grayish-pink and five gray eggs, but mostly dead.

March 5 (1907). All eggs are dead.

Lot 4: Treatment, two minutes. Acid in concentrated solution. Lot of sixty eggs. Treated June 20 (1906).

June 27, three eggs, partly gray.

August 28, eight gray; these and most of the yellow eggs still alive.

March 5 (1907), all dead.

Lot 5: Treatment, two minutes. Acid in concentrated solution. Lot of 250 eggs. Treated June 20 (1906).

June 27, five or six gray or cherry eggs.

August 28, fifteen cherry eggs; two gray, a few of which are alive.

March 5 (1907), all dead.

Lot 6: Treatment, one minute. Acid in concentrated solution. Lot of 205 eggs. Treated June 25 (1906).

June 27, one grayish egg.

August 28, three gray, eleven cherry eggs. Almost all alive.

March 5 (1907), all dead.

Lot 7: Treatment, one minute. Acid in solution. Lot of 140 eggs. Treated June 25, (1906).

June 27, two gray eggs.

July 1, fourteen gray eggs, mostly alive. Most of the yellow eggs also alive.

March 5 (1907), all dead.

Lot 8: Treatment, one half minute. Acid in solution. Lot of fifty eggs. Treated June 27 (1906).

July 1, two cherry eggs.

August 28, five gray eggs; several cherry, mostly alive. About two thirds of the yellow eggs also alive.

Lot 9: Treatment, one half minute. Acid in solution. Lot of ninety eggs. Treated June 27 (1906).

July 1, eight cherry or grayish eggs.

August 28, twenty-seven gray or pink-gray eggs of which

only two or three are collapsed Of the sixty or more yellow eggs, only six or seven are collapsed.

March 5 (1907), four live gray eggs; all others dead.

The treatment with glacial phosphoric acid seems to have the curious effect of prolonging the life of all the eggs whether they begin actual development or not, and of *slowly* initiating development in a considerable fraction of them, a proportion distinctly above the average number that would begin development without artificial stimulus.

Treatment by Sulphuric Acid. — Sixteen lots of unfertilized eggs and two of fertilized (as controls to indicate possible injury by the reagent) were treated with concentrated sulphuric acid for periods varying from one fourth of a minute to two minutes, and then washed with water. This acid is, of course, a strong dehydrator. In several cases only part of a lot would be treated, the other part left untreated as a check lot. The fertilized eggs developed normally and hatched, showing that the concentrated acid applied for two minutes does not injure the eggs. In all the treated unfertilized lots the proportion, above the normal average, of developing eggs was materially increased. This is also true of the treated parts of lots as compared with the untreated.

For example, in lot 2, a large lot of four hundred and fifty eggs, one hundred and fifty were treated and three hundred left untreated. In seven days ninety of the treated eggs were gray, while only five of the untreated eggs were gray. In lot 3, one hundred and forty eggs, one hundred were treated and forty left untreated. In ten days more than half the treated eggs were gray and alive, while none was gray in the untreated part. On the average from thirty to fifty per centum of the eggs in treated lots or fractions of lots began to develop, while in untreated parts of lots the per centum of developing eggs was less than ten.

Treatment by Hydrochloric Acid. — Twenty-four lots of unfertilized eggs treated with concentrated hydrochloric acid or with ten per centum hydrochloric acid, for periods of from one fourth minute to two minutes, then washed with water. The acid has but little dehydrating effect. On the whole the results show the distinctly stimulating effect of the acid, but some lots behaved aberrantly and the proportion of developing eggs did not go

beyond thirty per centum, and was usually not more than twenty or twenty-five per centum. The eggs treated with concentrated acids for the shorter periods, *i. e.*, one fourth and one half minute, were in better condition than those treated for one or two minutes. The eggs treated with ten per centum hydrochloric acid showed no special stimulation.

Treatment with Absolute Alcohol. — Killed the eggs.

Treatment with Potassium Hydroxide — Eleven lots of unfertilized eggs treated with strong solution of potassium hydroxide for periods ranging from one fourth minute to two minutes. All the eggs treated were loosened from their resting place and soon collapsed. Before dying the eggs showed a reddish color like the normal cherry of developing eggs, but from the great prevalence of this color in all the treated lots and parts of lots I am inclined to believe this color due to some special effect of the reagent on the egg shell rather than the indication of development. In the lots treated with strong solution for two minutes death and collapsing soon occurred, and in the lots and parts of lots treated for one fourth minute with half strength solution, collapsing occurred before it did in untreated lots and parts of lots.

Treatment with Lime Water.— Five lots of unfertilized eggs were treated with saturated lime water for periods varying from three minutes to one hour. No increase in proportion of developing eggs. The eggs of a fertilized lot treated with lime water for three minutes; all (except the small normal per centum) developed and hatched.

Treatment with Glacial Acetic Acid.— Seven unfertilized lots treated with glacial acetic acid, strong and half strong, for periods of one minute. Behavior of the lots uneven. In three of the lots no stimulating effect was noticeable. In two about ten per centum of the eggs developed. In one about thirty per centum began development, while in lot 7, a lot of three hundred and twenty-five eggs, half of which were treated and half left untreated more than fifty per centum of the treated eggs began development, while in the untreated lot very few, not more than two per centum. In a fertilized lot treated with the acid for one minute, all the eggs, except four or five, developed and hatched.

Treatment with Ammonium Hydroxide.— Six unfertilized lots

treated with strong ammonium hydrate for periods of one half minute or one minute. In two lots there was a beginning development of one third of the eggs; in the other four lots no increase over the normal average. In one of the two lots showing stimulation some of the eggs were left untreated and the increase in proportion (reaching thirty-three per centum) of the developing eggs occurred only in the treated portion of the lot. In the untreated portion only four per centum of the eggs began development. A fertilized lot treated with the reagent developed and hatched normally.

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THE EARLY DEVELOPMENT OF THE LATERAL LINE SYSTEM OF AMIA CALVA.[1]

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The earliest stage of the lateral line system of *Amia* described by Allis ('88) is in an embryo a day after hatching. In this stage the supra-orbital, infra-orbital, opercular-mandibular and the post-auditory lines are well formed but no individual sense organs are differentiated. Wilson ('91) has described for *Serranus* a common anlage for the lateral line, auditory organ and branchial sense organ. An elongated furrow formed from the inner layer of the ectoderm lies on either side of the neural tube. This furrow becomes divided by transverse constrictions into anterior, middle and posterior parts. The anterior and middle parts are transformed into vesicles by the closing together of the lips of the furrow and become respectively the branchial sense organ and the auditory organ. The posterior part remains as a groove and is converted into the lateral line.

Wilson and Mattock ('97) describe for the salmon a thickening in the form of a solid cord which behaves exactly as the furrow in *Serranus*. It is constricted into three parts, the two anterior of which form vesicles, while the posterior remains as a cord which is the anlage of the lateral line. Mitrophanow ('93) has described a similar condition in selachians. In this case there is a shallow furrow which merges gradually into the surrounding tissue, thus giving a less distinctly defined groove than in *Serranus*.

The present work was begun with the purpose of tracing the lateral line system in *Amia* from its first appearance up to the point where Allis took it up (*i. e.*, in embryos a day after hatching). It was thought possible that the anlage was formed and differentiated in *Amia* as described for teleosts by Wilson and Mitrophanow. The work was done at the Zoölogical Laboratory of the University of Michigan in 1900, under the direction of Professor Jacob Reighard, to whom I wish to express my sincere thanks.

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 114.

In embryos in which the optic vesicles and hind brain are just being differentiated from the neural tube there is seen in surface view a thickening (Fig. 1, *th.*) on the neural tube just back of the hind brain. This thickening appears to be a lateral extension of either side of the neural tube and is very similar in appearance to the optic vesicles. It was thought that this might be the anlage for the lateral line. This view was made more probable by the fact that in a later stage (Fig. 3, *th.*), when the first gill slit (spiracular) is formed, this thickening becomes divided on either side by a transverse fissure into two lobes, the cranial one of which lies opposite the hyoid arch. This bilobed condition suggests the division spoken of by Wilson in teleosts.

Sections (Fig. 2, *n.c.*) through the region of this thickening in the first stage described (*i. e.*, Fig. 1) show the neural tube still connected with the ectoderm while a wedge-shaped mass of closely packed cells, or neural crest, lies on either side of the tube between the ectoderm and the dorsal surface of the tube. Laterally these wedge-shaped masses project out beyond the neural tube. The lines of separation between this very much thickened neural crest and the neural tube on one hand and the ectoderm on the other are very indistinct. This neural crest corresponds exactly in position and extent to the external thickening described for the stage shown in Fig. 1.

In sections through the anterior half there is on either side of the crest the deep auditory invagination of the inner layer of ectoderm. The invagination is directed ventrally and inward toward the neural tube. The medial side lies against the broad end of the wedge-shaped neural crest. The wall of the auditory invagination consists of a single layer of columnar cells. The two walls are separated from each other by a plane extending from the outer layer of ectoderm to the inner surface of the invagination (Fig. 2, *a.in.*). Sections toward the anterior end and middle of the crest show that the auditory invagination ends abruptly in both cranially and caudally.

In a little older embryo (Fig. 3) sections show that the neural tube has lost its connection with the ectoderm. The neural crest has become divided into an anterior and posterior lobe as described in surface view and is not so clearly defined as in the pre-

vious stage, since the cells are more loosely packed and the cells of the ventral and lateral surfaces merge gradually into the surrounding mesoblast (Fig. 4, *n.c.*). On account of this pushing apart of the cells the crest extends farther ventrally along the sides of the neural tube. Laterally it extends out on either side to a distance equal to the width of the neural tube. The auditory invagination lies between the two lobes of the crest. A cavity has appeared in the auditory invagination in the form of a slit separating the two walls along the plane spoken of in the previous stage. A constriction separating the invagination from the ectoderm is in process of formation.

In a stage in which two gill slits (spiracular and first post-hyoidean) are formed (Fig. 5) the two lobes of the neural crest seen in surface view of the previous stage are no longer apparent in surface view. Sections show that the lobes of the neural crest have extended further laterally and at the same time thinned out dorso-ventrally so that they are transformed into two narrow bands of mesoblast extending outward and forward from the neural tube, one into the hyoid arch and the other into the first branchial arch. The auditory invagination lies at the median end of the post-hyoidean slit between the ends of the hyoid and branchial arches and extending into the mesoblast of the posterior edge of the hyoid arch. The constriction observed in the last stage between the ectoderm and the auditory invagination has proceeded until the auditory invagination is now a closed vesicle nearly separated from the ectoderm (Figs. 5, *a.p.*; 6, *a.in.*).

This auditory invagination was traced in sections of later stages until in a stage like Fig. 7 it has become a large vesicle, circular in section, lying some distance below the ectoderm. Its median half is partly covered by the hind brain so that only the lateral portion is visible in surface view. Its wall is composed of a single layer of cells which are more columnar on the median side than on the lateral.

The thickening on the neural tube proves then to be a mass of mes-ectoblast in the form of a neural crest in which the auditory invagination lies and so cannot be the anlage of the lateral line system. The neural crest of this region divides into two lobes which go in large part to form the mesodermal portion of the

hyoidean and the first branchial arches. It also probably gives rise to the seventh and ninth cranial nerves. In the region of the cranio-lateral portion of the neural crest there is on each side the auditory invagination of the ectoderm. This becomes constricted from the ectoderm and forms a closed vesicle or auditory vesicle. As the auditory vesicle undergoes no further division it is evident that the lateral line anlage does not arise in connection with the auditory organ as described in teleosts. The beginning of the lateral line system appears in a later stage.

The first indication of a lateral line is found in sections of a stage long after the establishment of the auditory vesicle (Fig. 7) and about a day and a half older than the stage represented by Fig. 5. A thickening of the inner layer of ectoblast caused by the cells becoming columnar runs along each side of the embryo in the angle which the embryo makes with the yolk (Fig. 8, *p.a.l.l.*). It extends from the second gill slit to the region of the first somite. It is not visible in surface view. This proves to be the anlage of the post-auditory division of the lateral line system.

In embryos about a day and a half older than that last described the lateral line is first seen in surface view (Fig. 9, *p.a.l.l.*). The post-auditory anlage has elongated so as to extend to the middle of the second post-auditory somite. It is a very slender cord formed from the ectodermal thickening described in the previous stage. It is of greater depth than in the earlier stages, so that its outer surface is slightly raised above the surface of the body. The anlage of the head lines also appear in this stage (Fig. 9, *s.o.l.l.*, *i.o.l.l.*, *o.m.l.l.*). The supra-orbital and the opercular-mandibular lines form a V, the arms of which extend craniad from a point just in front of the auditory organ. The supra-orbital extends to the caudo-dorsal portion of the eye and consists of two elongated bead-like thickenings end to end. The opercular-mandibular extends down the cranial edge of the gill cover and is a very slender line. Passing from the caudo-ventral portion of the eye and into the angle of the V is the infra-orbital line which is a short broad thickening. The lines in the head region are formed by thickenings of the ectoblast which project inward very slightly, but also raise the surface in the form of ridges. The thickenings are caused here also by the ectoderm cells becoming columnar (Fig. 10, *i.o.* and *s.o.l.l.*).

In an embryo that has just hatched (Fig. 11) the supra-orbital line extends cranially to a point just dorsal to the eye and is a continuous cord instead of being beaded. The infra-orbital line extends below the eye to the ventral surface of the nasal pit. The opercular-mandibular has changed but little from the condition in the previous stage. The post-auditory line has grown craniad so that the anterior end extends to about the middle of the auditory pit. Caudally it extends to a point half way between the operculum and the pectoral fin. This line consists in this stage of three elongated bead-like thickenings placed end to end.

The connection of the post-auditory line with the infraorbital line shows first in an embryo a day after hatching (Fig. 12). This is the stage with which Allis begins his description. The post-auditory line extends caudally to the pectoral fin and shows a fine beaded appearance. The head lines are very much broader than before and the supra-orbital extends farther over the eye. The infra-orbital sends a small twig up over the nasal pit. The line connecting the infra-orbital with the post-auditory line is a very fine cord. A very small twig is given off from this line just below the auditory organ and projects half way up the caudal border of this organ. There is no differentiation of the lines into definite sense organs in this stage.

Summary.

1. In very young embryos there is present a thickening on the neural tube which consists of a mass of mes-ectoblast of neural crest origin. In this the auditory invagination lies embedded. This portion of the neural crest later becomes divided into two lobes between which the auditory organ lies. In a still later stage the two lobes have become thinned out into sheets of mesoblast which extend one into the hyoid gill arch and the other into the first branchial gill arch. The seventh and ninth cranial nerves are probably also formed from the neural crest. The intimate relation of the auditory invagination to these lobes of the neural crest produces a striking resemblance to the condition found in the sensory anlage in teleosts. Here a single elongated thickening is described as dividing by two transverse fissures to form the anlage of the branchial sense organ, the auditory organ and the lateral line system respectively.

2. The auditory and lateral line organs of *Amia* do not have a common anlage. The auditory organ arises before the lateral line and independently of it as an invagination of the nervous layer of the ectoderm and forms later a closed vesicle.

3. The lateral line appears first in an embryo in which the auditory organ is a closed vesicle. The four primary lines (supra-orbital, infra-orbital, opercular-mandibular, and post-auditory) all arise independently of one another and of the auditory organ as thickenings of the nervous layer of the ectoderm and unite later to form a continuous system.

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'93 Étude Embryogénique sur les Selachiens. Archives de Zool. Exp. (3), I. 160–220, Pl. IX.–XIV.

Explanation of Plate I.

Fig. 1. Embryo of *Amia* in which there is a thickening (*th.*) of the mesectoderm or neural crest on the neural tube just back of the hind brain. × 20.

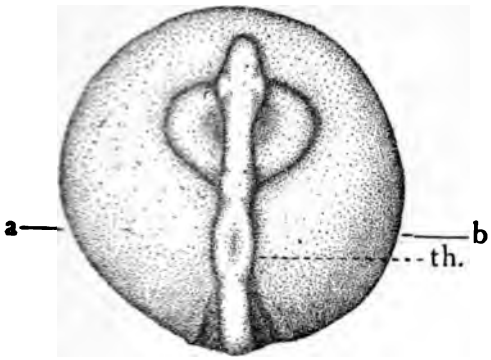
Fig. 2. Section through thickening along the line *a–b*, Fig. 1 showing neural crest and auditory invagination. *a.in.*, auditory invagination; *ec.*, ectoderm; *en.*, entoderm; *n.c.*, neural crest; *n.t.*, neural tube. × 167.

Fig. 3. Embryo of *Amia* showing the division of the thickening (*th.*) into two lobes. × 20.

Fig. 4. Section along the line *a–b*, Fig. 3, showing the gradual merging of the neural crest into the surrounding mesoblast. *ec.*, ectoderm; *en.*, entoderm; *n.c.*, neural crest; *n.t.*, neural tube. × 167.

Fig. 5. Embryo of *Amia* showing the extension of the neural crest into the spiracular and first branchial arches. *ad.org.*, adhesive organ; *a.p.*, auditory pit; *1st b.a.*, first branchial arch; *h.a.*, hyoid arch; *m.a.*, mandibular arch. × 20.

Fig. 6. Section through line *a–b*, Fig. 5, showing the constriction of the auditory invagination from ectoderm. *a.in.*, auditory invagination; *ec.*, ectoderm; *en.*, entoderm; *n.t.*, neural tube; *ntc.*, notochord. × 167.


a
b
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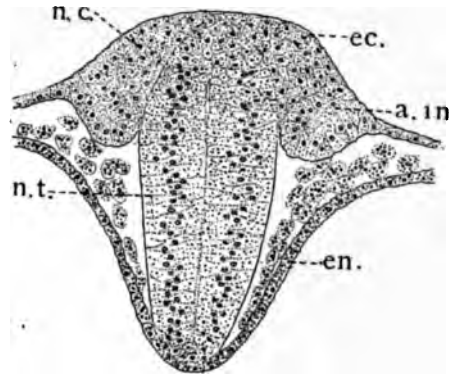
1


n. c.
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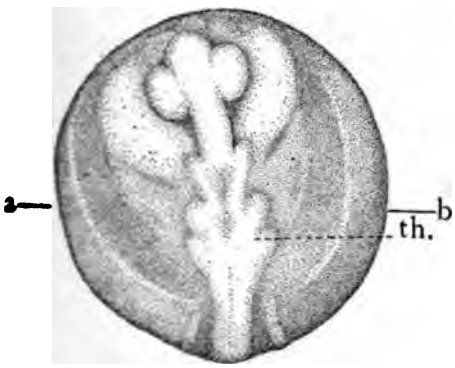
2


a
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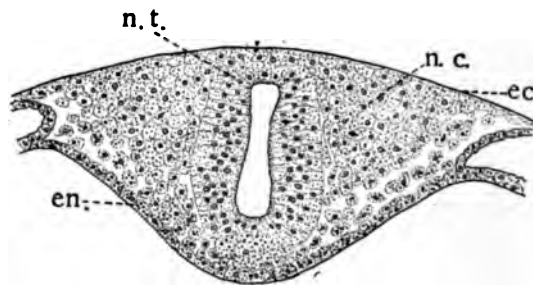
3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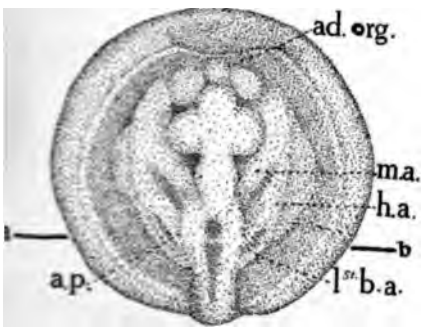
ad. org.
m.a.
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b
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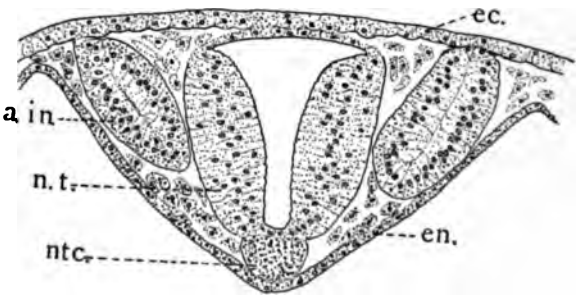
1st b.a.

5


ec.
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ntc.
en.

6

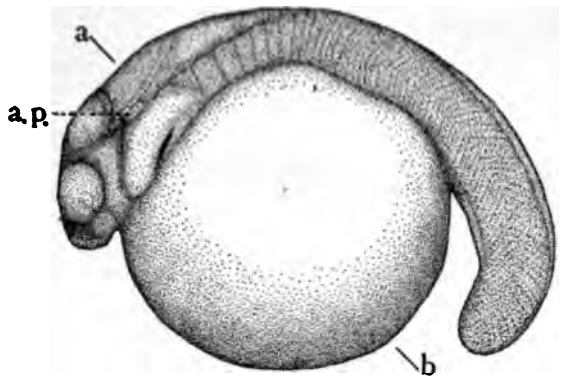
EXPLANATION OF PLATE II.

FIG. 7. Embryo of *Amia* in which the lateral line anlage is first visible in section but not in surface view. *a.p.*, auditory pit. × 20.

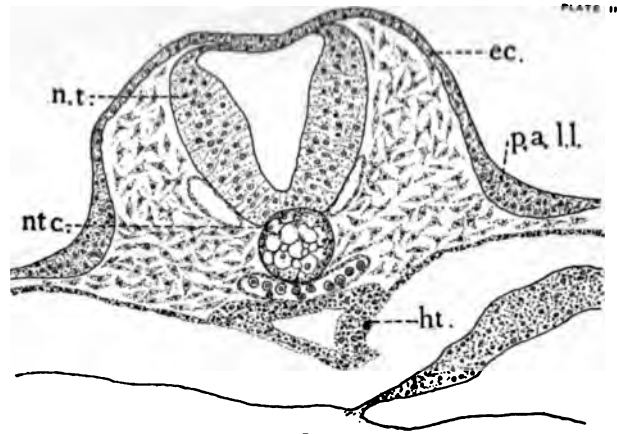
FIG. 8. Section along line *a–b*, Fig. 7, showing the first appearance of the anlage of the lateral line. *ec.*, ectoderm; *ht.*, heart; *n.t.*, neural tube; *ntc.*, notochord; *p.a.l.l.*, post-auditory lateral line. × 167.

FIG. 9. Embryo of *Amia* showing the auditory pit. The lateral line anlage is visible as ridges, in both head and body regions. *a.p.*, auditory pit; *i.o.l.l.* infra-orbital lateral line; *o.m.l.l.*, opercular mandibular lateral line; *p.a.l.l.*, post-auditory lateral line; *s.o.l.l.*, supra-orbital lateral line. × 20.

FIG. 10. Section along line *a–b*, Fig. 9, showing the thickening of the inner layer of ectoderm to form head lines. *ec.*, ectoderm; *i.o.l.l.*, infra-orbital lateral line; *n.t.*, neural tube; *op.v.*, optic vesicle; *s.o.l.l.*, supra-orbital lateral line. × 167.


a
a.p.
b

7


ec.
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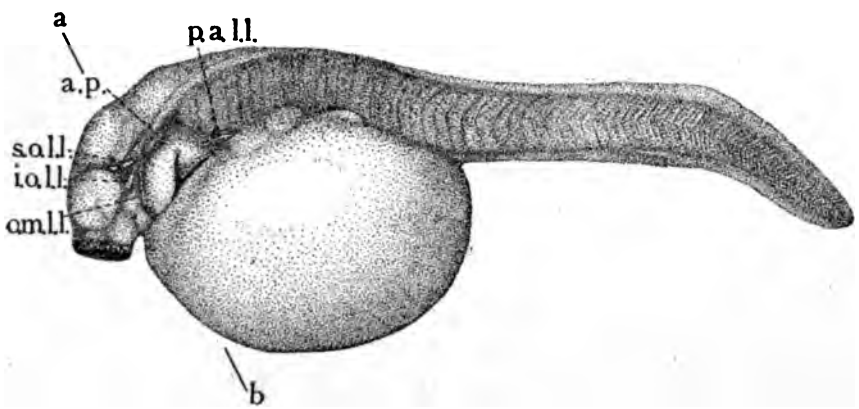
nt c.
ht.

8


a
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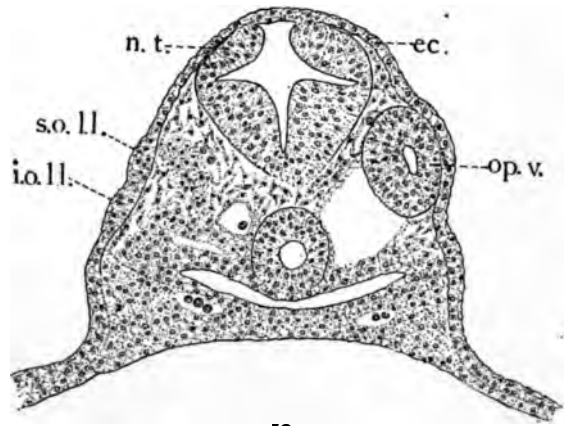
9


n. t.
ec.
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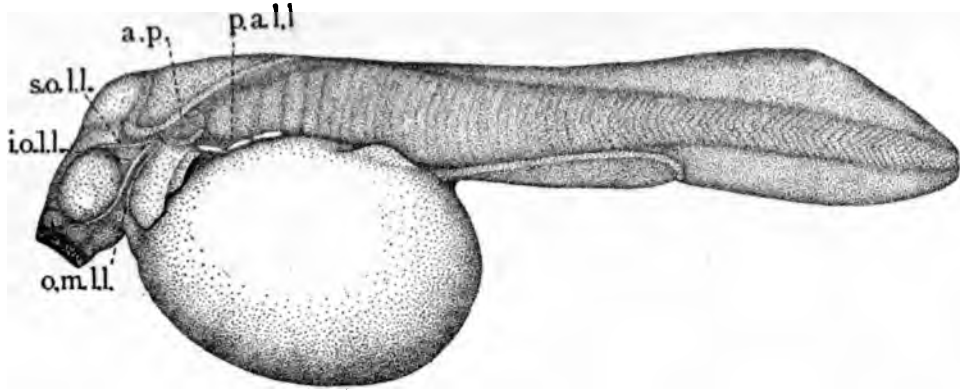
i.o.l.l.
op. v.

10

Explanation of Plate III.

Fig. 11. Embryo of *Amia*, newly hatched, showing further development of the four primary lateral lines. *a.p.*, auditory pit; *i.o.l.l.*, infra-orbital lateral line; *o.m.l.l.*, opercular-mandibular lateral line; *p.a.l.l.*, post-auditory lateral line; *s.o.l.l.*, supra-orbital lateral line. × 20.

Fig. 12. Embryo of *Amia*, one day after hatching, showing the four primary lateral lines before definite sense organs are differentiated. × 20. *a.p.*, auditory pit; *i.o.l.l.*, infra-orbital lateral line; *o.m.l.l.*, opercular mandibular lateral line; *p.a.l.l.*, post-auditory lateral line; *s.o.l.l.*, supra-orbital lateral line.



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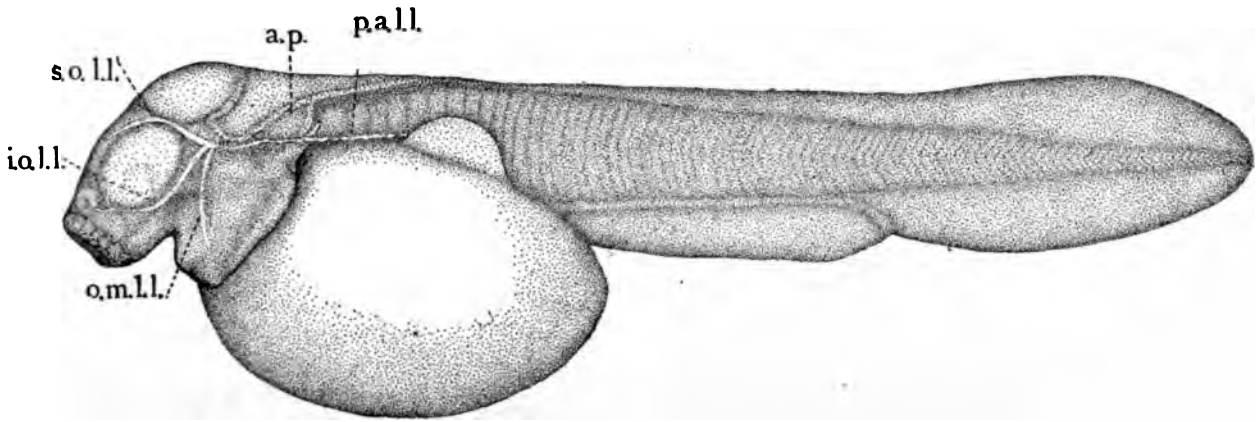

12

THE BREEDING HABITS OF THE RAINBOW DARTER (ETHEOSTOMA CŒRULEUM STORER), A STUDY IN SEXUAL SELECTION.

CORA D. REEVES.

Contributions from the Zoölogical Laboratory of the University of Michigan. No. 113.

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I. INTRODUCTION.

The brilliant coloration of the rainbow darter has been frequently mentioned since its first description by Storer (1845). It has given the fish its name and its reputation as perhaps the gaudiest of American fresh-water fishes (Jordan and Everman, 1896). The bright colors are present, however, only in the male so that there is marked sexual dimorphism. A similar condition exists, so far as I can learn, in all the closely allied forms of darters, each showing a characteristic color pattern. In addition to this the males of each of the many species show in the breeding season a more intense coloration. In the spring of 1906 I discovered this *Etheostoma* breeding under conditions favorable for close observation, and undertook the following study in order

to learn if possible whether any relation could be found between the behavior and the color characters. The work was carried on under the supervision of Professor Jacob Reighard to whom I am indebted for many suggestions.

The only published account that I have been able to find of the breeding habits of the darters is a brief notice by Seal (1897) who observed *Boleosoma olmstedi* and *Etheostoma cœruleum* in the Washington Aquarium. The rainbow darters observed by Seal were probably not kept under normal conditions for spawning, for in an aquarium the water is usually too deep, there is no current, and the available spawning area is small. My own observations were made in Mallet Creek about three miles east of Ann Arbor, Mich., where the stream is three to six feet across and forms a succession of shallow rapids and deep pools, varying in depth from two inches to two feet.

II. Usual Appearance, Habitat, and Behavior of the Fish.

Etheostoma cœruleum seldom exceeds two and one half inches in length, including the caudal fin. Among the fish examined there was little difference in the size of the two sexes, although a few males of three inches were found while no female reached that length. The colors of both sexes are described by Jordan and Everman (1896) and other systematic writers.

The colors of the female only slightly change with age or season and are like the sand or the gravel upon which the fish rests. There is on the back and sides a background of tan or olive with brown or blackish patches. On the anterior part of the body these are small, indefinite, and irregularly placed, but along the lateral line they are closer together elongated, and obliquely directed. Near the front edge of the second dorsal they merge into transverse bars which are more distinct below the caudal part of the lateral line. There are usually five of these. The last bar has a projection backward which separates two light brown spots at the base of the caudal fin. The pectoral, ventral, and anal fins are nearly colorless or light yellow; the second dorsal has fine dashes of brown and yellow arranged in irregular longitudinal rows. The first dorsal has more distinct colors in the breeding season when the outer margin is of dull grey with an irregular

yellow bar beneath; a row of oblique brown dashes separates this from the grey of the lower half of the fin.

The young males are described (Jordan and Everman, 1896) as variously marked. Those sexually immature look like the females. The fully developed males, when not breeding, show the general pattern already described for the females but have the transverse bars on the posterior part of the body of bluish tint and have pale reddish orange spots between these. Varying amounts of peacock blue may be found on the cheeks and the ventral, anal, and caudal fins. The second dorsal is made conspicuous by two broad bands which extend the length of the fin;

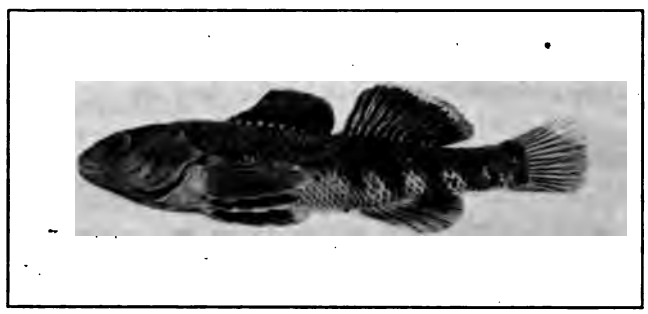

FIG. 1. The male *Etheostoma cæruleum* Storer, as he turns to display his ventral fins to another male. Photograph of a living fish slightly retouched.

the upper is of dull brick red while the lower is of peacock blue. The first dorsal is much like that of the female but with colors more intense since peacock blue takes the place of the dull grey of the outer margin. Over the belly and throat are patches of salmon or pale orange.

According to Jordan and Everman (1896), this *Etheostoma* is a Mississippi Valley form and the most abundant darter of the Ohio Valley. During most of the year these fish lurk among the stones and gravel of the small streams they inhabit. Their habitat is swift water. They are found at the lower ends of the rapids where the water enters the pools along the course of the stream, and also among the stones of the rapids themselves.

Living thus in exposed places the darters are very alert. If one approaches the stream cautiously, so as to remain concealed

until close to the edge of the rapids, he may see the startled fish flee by quick short dashes and sudden turns, and then they disappear. By carefully examining the bottom they may now be found lying in sinuous curves on the gravel or nearly hidden under the edges of stones with only a small part of the head or tail in sight. Here they pose at any angle to the current. They are often seen peering among the stones for food while supported on the pectoral fins so that the head and front part of the body are lifted above the bottom. The large pectoral fins are used both for progression and in directing movement. The second dorsal is usually elevated and with the anal presents a broad surface to the water when the tail is used in swimming. This very likely helps in their rapid, jerky, forward rushes which more resemble the leaps of a cricket than the graceful movements of a fish. The sudden darts and quick turns may serve to confuse an enemy, and to allow the fish to make off. But when no shelter is available the darter assumes a death-like quiet, and is then nearly invisible on the gravel bottom.

The shyness of the *Etheostoma* was shown when a number were brought into the laboratory and placed in an aquarium. For weeks they would not eat while observed and when any one was near they lay motionless upon the bottom. In time they learned to come at once to the side of the aquarium when I approached to feed them. But even after six months they fled as though frightened when a stranger came near or when I appeared in light colored clothing to which they were not accustomed. They seem to be capable of as much discrimination as the dace (Washburn and Bentley, 1906), but like young *Necturus* (Whitman, 1899) they are extremely timid so that it is a long time before they show their intelligence by their behavior.

III. The Breeding Colors, Habitat and Behavior.

With the coming of the breeding season [1] a change takes place in the rainbow darters as to color, habitat, and activities. As stated, the females retain their usual colors. They are somewhat

[1] The breeding this year (1906) was observed from April 24 to June 2. It is probable that the season began before my first records, as the males in the brooks had their bright colors the second week in March.

darker than later in the season, but are inconspicuously mottled with dark colors on an olive or brown background. In the young male the first dorsal often shows a dark blue color, the first bright color to appear. But the breeding males (Fig. 1) take on brilliant shades of red, orange, blue and green. As Holt (1898) states for *Callionymus*, so also in the male of *Etheostoma*, the back retains its mottled brown appearance, while the color pattern of the sides and fins becomes very brilliant. The blue of all parts becomes more intense and is of a bright peacock shade except where apparently darkened by black as in the bars and the ventral fins. The reds also become in places a bright orange. The most brilliant colors were observed the first part of the season. Besides these seasonal changes, there are many individual variations. The blue of the first dorsal may cover the whole fin so as to obliterate the pattern. The blue bands across the posterior part of the body may be six or four instead of five; the red which alternates with these may form intervening bands or may occur in patches above and below the lateral line; the caudal fin which is sometimes light greenish blue may have two pale red or yellow lines radiating from the base of the fin. In addition to individual variations and seasonal changes there are changes in color tone which occur from day to day or from moment to moment. (1) The ground color in both sexes varies in tone with the color of the bottom. Thus it is dark on an ooze covered bottom, but when the high water sweeps away the ooze and exposes the underlying yellow sand the color of the fish becomes lighter in harmony with the bottom. (2) The colors are more brilliant when the temperature of the water is low. This change of color with decrease of temperature does not appear to coincide with increased spawning activity, since spawning was not observed when the water was below 15° C. The brilliant colors coincide rather with the low temperatures of the early part of the season before the actual spawning. Besides this the brilliant colors of the males, especially the blues often flash out momentarily and fade again when the rivalry between them is most intense, as described below.

In the spring the darters leave their lurking plaees in the rapids and congregate on the gravel sheets which are spread out at the

head of the rapids where the stream leaves a pool. Here the water is from one and one half to four or six inches deep with a current moving at the estimated velocity of about 75 feet per minute. The pebbles of the bottom are small, averaging one half inch in diameter while the largest is not over two inches. As these gravel sheets are the areas used by the dace (*Semotilus atromaculatus*) and stone-rollers (*Campostoma anomalum*) in nesting, the ground is often roughened by their pits and ridges.

FIG. 2. The breeding ground of *Etheostoma cæruleum* Storer. The stream is here about six feet wide. The stone at *1.* is about a foot long. The numbers indicate the holding of fish "A."

The change in the behavior of the darters is as marked during the breeding season as the change in color or habitat. While the fish are at other times shy, rushing for shelter on one's ap-

proach or lying so still as to escape notice, they now make no effort at concealment. It is often possible by nearing the stream slowly to reach the margin without frightening them. They quickly become accustomed to one's presence and are then not disturbed by one's wading among them. I have touched them with my boot tips or stroked them with a small wire without their moving. It is then possible to stand directly over them and even to examine them with a hand lens without in any way modifying their normal behavior. They appear to swim here and there at random.

The breeding areas so swarm with them that, one day, I counted twenty-six in a single square yard. Since the females are so inconspicuously colored as to be easily overlooked, there may have been more fish in that space. There always seemed to be more males than females present on the spawning ground. Early in the season I estimated four or five to one, and among my notes for June 1 is the item, "Seven darters in sight; only one female." This proportion continued for several days but collections of fish made in November did not show this inequality in the number of the two sexes. It therefore appears that the larger number of males on the breeding grounds depends on the difference in the habits of the two sexes as is shown below.

IV. The Spawning.

Although all the fish seem to be moving about over the spawning area promiscuously, close watching shows that this is true only of the females and small males. On the other hand, there are some of the large brilliant males which remain each within a restricted area which he guards and from which he drives the other males. Among such males it often happens that a single individual may be distinguished by peculiarities of coloration and may thus be kept under observation for hours at a time. The plots guarded by individual males we may call their "holdings." The width of these holdings does not usually exceed fifteen inches but as the length varies from fifteen inches to two feet they may include more than two square feet. A male may leave his holding to pursue a female or he may go beyond it while he energetically drives away another male, but in either

case he immediately returns. Each spawning area has a number of such holdings guarded by the large males.

1. *Behavior of a Large Male toward a Female.*

When a female enters the holding of a large male it rarely happens that the two are left undisturbed. Usually their behavior is greatly complicated by the interference of other males, but for the sake of clearness we may first consider what happens when the two fish are left undisturbed.

The female usually swims into the holding from below. The male then approaches her from behind as she lies on the bottom with her head up stream. He often places himself behind her, his body parallel with hers and with his snout touching her side near the posterior margin of the first dorsal. While in this position he may move his head with a trembling, vibratory motion. This vibration appears to have a rate of from four to eight per second and must cause a gentle tapping of the side of the female. The male may now move away from the female for some inches and upon returning, may place himself above the posterior part of her body and may then again move off for two or three inches and return. He becomes ever more excited in his movements and may at times place himself at right angles to the female an inch or so from her head and then vibrate his pectoral fins and head and elevate his gill-covers somewhat more than usual. This is not a very frequent mode of behavior but it may serve to display the colors of his cheeks, opercula and throat to the female. At any time the female may withdraw a few inches to one side. Once I counted ten such successive side-wise moves by a single female, each of two to four inches. The male followed after each move; sometimes he came near enough to tap her side, while at other times he lay at a distance — not exceeding three inches — and seemed to watch and to wait. If disturbed, as she sometimes is, by the vigorous taps or pokes of the male, the female may swim off for a few feet. To do this she often drops back and to the side, then turns and dashes up stream. She may thus escape pursuit by the male. On the other hand if the female starts directly forward while the male is near he follows very closely, since he appears to interpret the forward motion as the sign that

she is preparing to take the position for spawning. This whole process of stimulation by the male is manifestly adapted to excite the female to the spawning act.

2. *The Spawning Attitudes.*

When the female is ready to spawn she lowers her head and with her long axis at an angle of about 45° to the bottom she drives herself forward by vigorous strokes of the tail. This sends her head into the gravel. When she has succeeded in burying her head and the anterior part of her body beneath the gravel she depresses her tail so as to bring her long axis parallel to the bottom. This brings her head above the bottom but leaves her pectoral fins and the ventral portion of her body buried in the sand or gravel. The pectoral fins are extended at right angles to the body and add to the surface that is under the sand; they thus form an anchor which prevents the current from displacing the fish while the eggs are being deposited. If, either by the action of the water or by the movements of the males along the

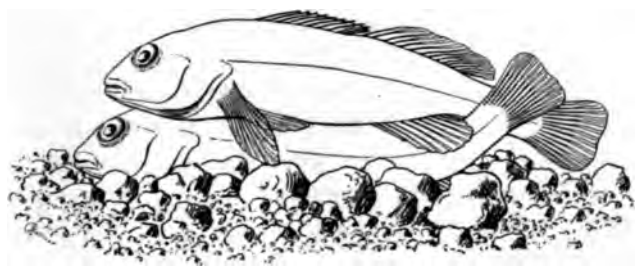

FIG. 3. Spawning attitude of *Etheostoma cæruleum* Storer, at the beginning of the act. Drawn by Mr. Carl Kellner from sketches by the author. Natural size.

sides of the female, her pectoral fins become uncovered; or if she is not successful in covering them when she attempts to bury herself, she moves a few inches trying again and again until her pectorals are fully covered. When the female has thus partially buried herself the male, recognizing her readiness to spawn by her behavior and attitude, may at once place himself above and parallel with her as she lies half-concealed in the sand (Fig. 3). His ventral fins are extended along her sides and rest against her body just in front of and below her first dorsal. His pectorals

are held nearly at right angles to his body often with their lower margins on the gravel. His anal fin is extended on one side of the female close against her body while his caudal fin is applied to her tail on the side opposite his anal. The position of the male is largely maintained by the firm hold given by pressing his ventral fins against the rough ctenoid scales of the female, while the backwardly directed spinules on his scales hinder his slipping backward and the spinules on her scales keep him from slipping forward. The crossing of the tail of the female by that of the male is frequently not maintained during spawning but seems to be the means by which the motion of the two fish is started in unison.

3. *The Spawning Act.*

When thus placed (Fig. 3) the male vibrates the head in the agitated trembling manner already described. This seems to be a stimulus for the spawning for usually the two fish begin at once a movement of the posterior portion of their bodies from side to side in unison. The motion is a very rapid vibration through only a short distance and lasts for several seconds. During this time the sand and gravel are stirred up and make a cloud which partially obscures the fish. At the same time the caudal fin of the male slips over that of the female so that it is on the same side of her body as his anal. That the eggs and milt are extruded at this time was proved by picking up the fertilized eggs immediately after the spawning had taken place. The eggs are adhesive and remain sticking to the gravel which forms the bottom of the depression in which they are deposited. Here they were found a number of times with the help of a reading glass, and were scooped up with the gravel in a bacteria dish and were found to be identical with those obtained by stripping the female. They are spherical, and measure about 1.5 mm. in diameter. They are pale yellow in color and contain a large oil drop. Only from one to six eggs were obtained at one time by stripping the females, but large individuals deposit probably twice or three times that number. Although the milt is of a milky color it is not shed in sufficient quantity to be seen in the cloud of sand stirred up by the rapidly moving fish.

Spawning may be repeated a number of times before the fish separate. Sometimes during spawning the fish, without separating, move forward along the bottom for short distances. In such cases, or whenever the anchoring of the female has yielded so that she is moved from the bottom by the vigorous activity of the two fish, she leaves the spot where she was lying and again buries herself before spawning is repeated.

4. *Behavior of Two Rival Males toward Each Other.*

Since the fish are crowded into small areas during the season of sexual activity, it is rare for a pair to spawn without a number of males crowding about the female. When a female enters the holding of a brilliant male, several males may attempt to follow her. As one of them approaches, the owner of the holding raises his first dorsal which is a very dark brilliant blue. Often at this warning a small male at once retreats, but if the fish are nearly equal in size and coloration the new comer may stand his ground and also make a display of his colors. With his first dorsal displayed by extending it until its anterior margin rests on his back and points forward, the defender of the holding may then turn on his long axis until his ventral surface is visible to his rival. He thus shows off its dazzling orange and the deep blue of the ventral fins. This attitude is shown in Fig. 1. While he is thus posed the two males frequently move to positions close to the female and parallel with her but on opposite sides. During this display the blue of the cheeks and ventral fins frequently flashes out and becomes darker. This momentary intensification of color was more noticeable early in the breeding season than toward its close.

If the intruder does not retire after this brilliant display he receives a series of blows administered by the defender of the holding who swims at his rival and strikes him repeatedly one fourth to one half inch back of the opercular region. Sometimes the blows are given by swimming directly into the opponent and using the head as a weapon, at other times by swimming past close beside him and giving a swift stroke of the tail. These combats from all appearances are as harmless as the color display but are in the end always successful in driving away in-

truders. They may be called sham combats. Occasionally the intruder resists. Then the two males range alongside each other, depress their heads nearly to the bottom of the stream, and with that peculiar trembling motion of the head already mentioned, and with synchronous movements of their caudal fins, swim about here and there side by side or move side-wise across the stream for short distances. The rivals when thus engaged rarely swim forward from the holding yet I have once seen two males swim forward for some ten feet, moving the caudal fins in unison while keeping so close together as to seem to touch one another. Professor Reighard tells me that he has observed a similar form of behavior to be habitual with horned dace (*Semotilus atromaculatus*).

5. *Behavior of Supernumerary Males toward the Spawning Pair.*

Young males frequently follow the female as she moves about in the breeding area. They approach, as already described, and tap her side; they often move forward so that the tapping is near her head. When thus disturbed the female swims off for a number of feet but does not often escape the small tormentors who swim up beside her and continue their vigorous stimulation. They are more agitated in their behavior than the larger males, especially during the latter part of the season. As the large male guards the female in the holding these young males often surround the pair. They lie off at a distance of six to eight inches and make frequent attempts to approach while the male in the center keeps up a nearly continuous chase to drive them away. During the spawning they often swim close to the side of the female moving the gravel from near her as they wedge themselves in at her side. When a number are present they may entirely cover the pairing fish. During the spawning the small accessory males that are in contact with the spawning pair move the body with the same vibratory motion and thus appear to take part in the spawning. They are not brilliantly colored. An examination of the milt showed them to be nevertheless mature. The spermatazoa were as active and when mounted in water their movements were as long continued as in the case of large, brilliant fish.

6. *Sex Recognition.*

Sometimes when a female slips out of sight of the male that has been guarding her, he, seemingly unaware of her absence, approaches a small male as if he mistook him for the female. As he approaches the young male the latter sometimes raises his lightly colored first dorsal as if to show his identity and then usually flees. In this case the large fish apparently fails to discriminate between the light-colored male and the female.

This failure was especially apparent in one instance when several males rushed from various distances and directions toward a young male that happened to burrow into the sand while feeding and thus took nearly the position of a female preparing to spawn. None of the males followed the young male as they would have followed a female, nor did they feed when they came near the spot where he had been, as they would have done if their approach had been due to the suggestion of food by the attitude of the young male. It thus seems probable that at a little distance the male fails to distinguish between dull males and females. The more nearly the behavior of a dull male simulates that of a female, as in the case of the male burrowing for food, the more is he likely to be mistaken for a female. Upon the near approach of the brilliant male the young male erects the first dorsal and rapidly escapes, modes of behavior not observed in the female. It appears then that the brilliant fish distinguishes between the two by their behavior; a mode of sex recognition pointed out by Holmes (1903) in the case of amphipods. In the case of very young males the sex recognition must be wholly of this character, while males which already show some little sexual coloration are probably distinguished upon near approach by means of it as well as by behavior. Holt (1898) believes that a similar method of recognition occurs in the dragonets.

V. Observations Bearing on Sexual Selection.

The preceding description of the breeding activities of the rainbow darters at once suggests that a field study of their behavior may be made to yield evidence as to the occurrence of sexual selection among them. The fish are crowded together in the breeding areas; the sexes are easily distinguishable; they may

be readily observed under wholly natural conditions; the breeding season is brief. Here if anywhere under field conditions it should be possible to determine whether sexual selection occurs.

I therefore attempted to observe continuously for as long a time as possible the behavior of individual fish in order to learn whether the more brilliant males succeed in spawning more frequently than those less brilliant, and whether individual females spawn more frequently with brilliant males than with those less brilliant. Individuality in color pattern has made it possible to thus follow and identify individual fish for several hours continuously. I have further noted in a large number of cases the colors of the pairing males in order to learn whether the more brilliant males are successful in a larger proportion of cases than the less brilliant males.

From a number of records in my notebook I have selected three of individual fish, that of a brilliant male (*A*), of a dull male (*B*) and of a female (*C*). An extract from my field notes of May 18, 1906, gives an average picture of the movements of a large male (*A*) that was under continuous observation for the latter half of the afternoon of that day. He had his holding on a gravel area just above a tiny rapid. The rapid was made by large stones and débris which obstructed the course of the stream and formed the lower margin of his area. Fig. 2 shows the breeding ground of this fish.

Fish A (Male).

4:05 P. M. A female is in the area with *A*. There is spawning.

4:10. Spawning is repeated; a small male rushes in beside the two fish. The female swims away with the small male while *A* remains near the spot marked No. 1 in Fig. 2.

4:13. *A* withdraws about a foot and begins to fight a male of his own size; both display their dorsal fins.

4:15. *A* is back at No. 1.

4:15½. He moves away less than a foot to where a female is lying. Spawning again 8 inches from the first place (No. 2 in Fig. 2). The female remains quiet while he drives off intruders.

4:18. She goes away with a smaller fish that rushes in by her side and pokes her. *A* remains near No. 2 and seems to be feeding.

4:19. The female returns.

4:19½. A small male rushes up to the side of the female. He pokes her so that she swims off but not far. *A* remains.

4:22. He moves about eight inches to where a female is lying. The two are under a riffle so they cannot be seen distinctly. (This spot is No. 3, Fig. 2.)

4:22½. *A* drives off a male.

4:25. He is guarding the female: he spreads his fins at the small male present at 4:19½. The female swims away and several males which have been near follow close after her.

4:27–30. *A* guards the holding.

4:31½. He seems to eat something near No. 2. He turns his head and searches quizzically among the stones.

4:34. Another female comes in but a small male is pursuing and she does not stay.

4:36. The female appears from under the riffle at No. 3 and buries herself as for spawning.

4:38½. She remains quiet while he guards her.

4:39. She moves to a spot in line between No. 2 and No. 3.

4:41. He drives off other males from the female for a space of a foot.

4:42. The spawning activity is begun but the female moves away and it is not completed. After this for several minutes no females are in sight. *A* moves about and once, when near No. 3, seems to feed and then he seems to spit something from his mouth.

4:51. A female slips into the area from below.

4:52. *A* approaches from behind and touches her side, while the two fish are in the following attitude $(\,)^{\varphi}_{A}$.

4:54. Spawning occurs; a small male tries to crowd in beside the female; she moves and leaves. *A* remains, guarding the spot where spawning has just occurred.

5:05. The female again enters and buries her head and the ventral part of her body. She is between No. 1 and No. 3. *A* drives away a small male.

5:08. He moves up to her head and seems to display himself to her. He drives away a small male.

5:10. She tries again to bury herself. *A* comes close to her, tapping her side.

5:11. She tries again to bury herself, again he taps her side in position here shown $(\,)^{\varphi}_{A}$.

5:12½. She buries herself, but again goes off and two small males follow her as she swims to a point some three feet away while *A* remains.

During the last twenty minutes of the above record the same female was under observation and it is probable that the same one was in the area earlier, but watching the male made it impossible to follow the female in each case. This brilliant male rarely left an area approximately two feet long and eight or ten inches wide. During the first hour there were in this area but four spawnings, in all of which this male took part. A comparison of his activities with those of a small dull male is instructive.

Fish B (Male).

After choosing an especially small, dull specimen the following notes were made, on May 22:

3:12 P. M. *B* is in area *X* (about a square foot in the center of the stream which looks like a favorable spawning area, and is a point from which to measure his movements).

3:14. *B* moves down stream for a short distance toward a female which is approaching *X*.

3:15. He is back in *X*.

3:15½. *B* moves toward the side of the stream and follows a female, but a large brighter male remains near her.

3:17. *B* is again in *X*.

3:18. *B* moves two feet back and to the side.

3:18½. *B* is in *X*.

3:21 P. M. He is driven away from *X* by a small but brighter male.

3:23. *B* moves out to the side 18 inches, then one foot in front of *X*; next 18 inches to the side of *X*. Every male seems to drive him away.

These records extend to 3:37½ and show continuous movements by *B* every one half to two minutes and extending in

every direction from *X* and over distances of from four inches to four or five feet. He raises his first dorsal occasionally as he is driven about. It seems possible that at a distance the other males mistake him for a female. While there is in the case of this male no record of spawning, notes of other days observations show that the small, dull males succeed occasionally in spawning. They sometimes rush into an area guarded by a brilliant male and spawn with the female, while the brilliant male is chasing away other intruders. They also often move close to the female and into the mantle of males which covers her while she is spawning. Yet relatively few times are these small males successful in spawning.

Fish C (Female).

From my notes of the afternoon of May 12, when watching a single female, I give a sketch of the hour from 4:15 to 5:15. At the beginning of this time a small, dull male poised near a female gave her four to six taps with his lower jaw just back of her first dorsal. She started to bury herself, then two bright males came, she moved forward about 18 inches, followed by a brilliant male with which she spawned after burying herself. A little later followed by a dull male she came near a brighter male and he pursued her for about two feet. A dull male came and tapped her side. She started and moved quickly forward and to the side two or three feet to where there was a large brilliant male. He drove off the small males, she buried herself and the two spawned. Two other males crowded in at her side during the spawning. After spawning the fish were quiet for a few moments when the female moved to the shelter of a stone and remained quiet about one half hour. She then moved to the opposite side of the stream and was quiet again. At the end of the hour a small dull male tapped her side, she moved forward, buried her pectorals and a medium sized dark colored male spawned with her. Thus in a single hour this female spawned three times, each time with a more brilliant male, while during the same period three attempts of small or dull males to spawn with her were frustrated at their beginning.

These are samples of the records of individual fish. I also

made a note of the pairings observed and in 57 cases recorded the color of the male. These results are arranged in the following table.

TABLE SHOWING THE COLOR OF THE PAIRING MALES IN FIFTY-SEVEN SPAWNINGS OF *Etheostoma cæruleum*.

Color of Male.	Number of Records from April 24 to May 18 Inclusive, 25 Days.	Number of Records from May 19 to June 2 Inclusive, 15 Days.	Whole Number of Records.	Per Cent. of the Whole Number.
Bright.	17	18	35	61.4
Medium.	3	5	8	14.0
Dull.	2	12	14	24.6
Total.	22	35	57	100.0

The largest males and many of the somewhat smaller ones are bright colored and are classed as bright. Others of the smaller males are not so bright and are classed as medium; the small fish with little color are classed as dull. If we divide the medium class between the bright and dull we have a ratio of 39 bright to 18 medium and dull or a percentage of about 68 to 32. This is perhaps as fair a statement of the results as can be made. The work, however, does not furnish conclusive evidence of selection because the results are complicated by the presence of supernumerary males. In 17 of the 35 cases recorded in the table for bright males there were from 1 to 6 of the smaller fish present. Though it is probable that they are less effective in the fertilization of the eggs than are the bright males, they cannot be neglected in the consideration of the problem. This is more evident from the fact that the brilliant males rarely take a position among the supernumerary ones. The table shows that the small males succeed more frequently toward the close of the season than at the beginning. If it were true that only the larger females spawn the first of the season then it would be relatively sure that there was selection by the pairing during this time of the larger and better developed fish of both sexes, but this is not the case because among the very first day's notes is the statement that the females spawning varied in size from those only about one third the size of the large males to those as large as the average fish. In spite of these disturbing factors, the larger percentage of cases in

which the brilliant males are successful, the fact that they are the largest and most vigorous and the advantage which results to them from their position during the spawning act, makes it probable that much the larger part of the eggs are fertilized by them.

VI. Discussion of Results.

1. *Origin of Nest-Building Habits.*

The facts here presented show clearly that certain large males have holdings which they guard and over which they remain, while the females and young males are moving about in the breeding area. The way in which these areas come to be held by the large males was not observed, but it seems possible that the result has been reached in the following manner: As the female moved about at the beginning of the season with the males following her, she attempted to bury herself, but owing to the hardness of the bottom was at times unsuccessful. She repeated her attempt until she came to a place where the bottom was loose, and where she easily worked herself into the gravel (*cf.* for Salmon, Rutter, 1903). Then spawning took place. When she moved she again succeeded in burying herself where the sand was loose. Since the large males were able to drive away the smaller ones, they appropriated to themselves those areas within which the bottom was of a character suitable for spawning. These were their holdings. When a male had once taken possession of a holding he received into it a succession of females and guarded it continuously against the intrusion of other males. In this way he not only secured to himself the successive females that visited his holding, but incidentally he guarded the eggs that had been deposited in it and prevented their being eaten by the females and by other males. By this method the breeding area may have come to have a number of holdings, each defended by its male.

In *Etheostoma* the male does not in any way prepare the holding for the reception of the eggs, but these are laid here and there at random wherever the bottom proves suitable. There is in this case no real nest. It has been noted that among certain fish which do in one way or another prepare a nest for the eggs, an

area of the bottom surrounding the nest is guarded (Reighard, 1903 and 1905) and so may be considered the holding. Thus the habit which the male of *Etheostoma* shows of guarding a limited area of the bottom may be regarded as more primitive than that of the nest-building fishes. It represents probably a stage in the evolution of the nest-building habit.

2. *Displays of Color and of Movement.*

There is a marked difference in the behavior of the males and females on the breeding area. The females are passive and, save as they swim off to avoid the males that crowd about them to stimulate them with their vibratory tapping, they make no response to the solicitation of the male, no displays of color and no responsive movements. Only once have I seen a female respond by a trembling movement of the head and pectoral fins. In all other cases the females appeared indifferent to the fish about them. In contrast to this is the behavior of the males. The large, brilliant males make less use of mechanical stimulation than do the smaller ones. As has been stated the larger males sometimes remain quiet at the side of the female for a considerable time while only a few inches distant and in full view. Occasionally one places himself at right angles to a female and, with his head about an inch from her's, elevates his gill covers and vibrates his pectoral fins. Whether either of these attitudes is for the purpose of displaying to the female the colors of the male is not easily determined. The younger, less brilliant males have never been observed to make any display of color and appear to rely wholly upon mechanical stimulation. They are more active than the older males and follow the females more persistently so that the vigor of movement shown by the males in courting may be said to vary inversely with the brilliancy of their colors. The rate of the vibratory movement of the head of the male has not been measured but was compared with the rate of the most rapid tapping which could be made by a slight movement of the fingers and was then estimated at from four to eight per second. As the lateral line organs are sensitive to such vibrations (Parker, 1903) it is possible that when the fish are not in contact the vibratory movements of the male are transmitted

through the water and affect the lateral line organs of the female. In any case it appears that mechanical stimulation forms the chief element in the behavior of the male toward the female while attitudes especially suited to display his colors are less evidently used.

Between the males color displays are frequent. If two brilliant males are rivals and of the same size they pose side by side with their first dorsals elevated. If one of the two fishes is a small male he does not pose, but after elevating his first dorsal flees. This display by the small male appears to serve as a sex recognition character by which the other males distinguish him from the females. Displays of force also take place between brilliant males. These consist of blows delivered by the tail or head of one male against the side of the other. Or they consist of sham struggles during which the fish swim about side by side. While thus swimming the first dorsal fin is always raised and each of the two fish makes use of the same vibratory movement of the head that is used toward the female. The net result of these displays of color and force between males is in the majority of cases to exclude the smaller males from participation in pairing. They appear to have, however, a further effect; that of raising the general state of excitation among the males. H. E. Zeigler is quoted by Gross (1896) as expressing the belief that a high state of nervous excitement is necessary for the pairing of all animals. Häcker (1900) has applied this interpretation to the displays of color and movement of male birds during the mating season. He believes that they serve to overcome the coyness of the female and to bring her into the physiological state necessary for pairing. The same interpretation seems to apply to the breeding behavior of the male *Etheostoma*. Were the displays of color and of force of the individual male merely random, not manifestly adjusted to a female in one case and to a male in another case, then they might be interpreted as the meaningless result of nervous excitation. But since they are exactly adjusted I conclude that they have, in addition to their function of limiting the breeding activities of the smaller males, a further definite function; that of raising the general state of excitation among all the participating fishes.

3. *Sexual Selection.*

The evidence has been already presented to show that in *Etheostoma* the bright males are most frequently successful in spawning. I would be justified, from some days' observations, in claiming evidence for sexual selection in the Darwinian sense, a selection of the brilliant males by the females. For my notes show that the females, although followed in their course by small males, often go directly from the holding of one large male to that of another. They thus appear to consciously neglect or repel the smaller and more insistent males and to give preference to the larger ones. On the other hand my observations show that the females spawn with whatever male happens to be present. Even in the holding of a large male the spawning is sometimes with a small one while the large one is driving away intruders. Hence, although the brilliant males are more successful in pairing than the duller males, yet I find no evidence that the female *chooses* them. What appears to be a choice of males is probably in reality a choice of spawning places. This subject has been discussed under the heading "Origin of Nest Building Habits." We then find no sufficient evidence in the behavior of the female that she so discriminates between males as to give to any color or color pattern selectional value in the sense of Darwin (1883). If this be true, the displays of color and movement (by the male before the female) result, not in a selection by the female of particular males but only in an increase of the general state of nervous excitement among the participating fishes. (Cf. for birds Häcker, 1900.) It does not appear that the female exercises any choice either conscious or unconscious based on color or movement.

The only form of selection that appears to be present is that which arises from the rivalry of the males and results in the limitation of the breeding activities of the smaller males. Since the smaller males undoubtedly breed when older the case is merely one of "seniores priores." The preponderance of the older males in the breeding cannot have resulted in the evolution of secondary sexual characters. This could have come about, by selection, only through the preponderance in the breeding of males having certain secondary sexual characters not possessed by other males.

It would seem that the more brilliant colors are of value to the males possessing them during their combats with other males. But whether the males that succeed in breeding really differ in any definite way in color or color pattern from adult breeding males in general, whether they belong to some one of the many types of coloration, my observations do not show.

Summary of Observations.

1. In the adult *Etheostoma cæruleum* the two sexes are distinguishable at all seasons of the year because the males have some red and blue in the color pattern while the females are mottled brown.

2. They usually inhabit the rapid water of small streams.

3. Out of the breeding season the fish are shy and not easily approached.

4. The breeding season was observed to extend from April 24 to June 2, 1906.

5. The sexual activity of these darters is limited by temperature and actual spawning was not observed when the water was below 15° C.

6. The colors of the larger males are more brilliant in the breeding season than at other times. The red and especially the blue are greatly intensified and appear on parts of the body on which they are not found during the rest of the year. The adult males show many variations or types of coloration.

7. The most brilliant colors were found the first of the season, or the last week in April.

8. The fish at this time congregate on the shallow gravel areas above the rapids.

9. They lose their shyness and allow close observation without showing signs of fear.

10. The sexes occur in equal numbers but there are always more males than females present on the breeding ground at one time.

11. Among these males certain large brilliant ones have holdings which they guard. They may leave these to drive away other males or pursue a female but they return promptly.

12. The driving away of rival males is by means of display or by blows of the tail or head.

13. The males occasionally display their colors to the female.

14. The males also tap the sides of the female by a trembling vibratory motion of the head.

15. Before spawning the females partially bury themselves with their pectoral fins covered by the sand or gravel of the bottom.

16. The male places himself above the female in the position shown in Fig. 3, and during a rapid synchronous vibration of the tails of the two fishes the eggs and milt are extruded.

17. The eggs remain adhering to the stones of the bottom of the very small depression made by the body of the female.

18. The spawning is frequently repeated by each female but only a few eggs are deposited at one time.

19. One or several males may be present during the spawning act.

20. When supernumerary males are present they take positions above the female and at her sides.

21. Large males seem to mistake the undifferentiated males for females and to distinguish them by behavior rather than by appearance.

22. The raising the first dorsal as practiced by the males seems to serve as a sex recognition character.

23. The brilliant males were successful in pairing in over 60 per cent. of the observed cases. The less brilliant males were thus excluded from the pairing in these cases, though they may have been present as supernumerary males.

MANISTEE, MICH.,
September 17, 1907.

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BIOLOGICAL BULLETIN

GILL DEVELOPMENT IN MYTILUS.[1]

EDWARD L. RICE.

In the present paper no attempt is made to give an exhaustive treatment of the subject; the intention is rather to summarize briefly the work that has been done upon the development of the lamellibranch gill, and to describe somewhat fully certain phenomena in the development of the gill of *Mytilus* which seem thus far to have escaped notice. A few incidental observations on gill development in other lamellibranch genera are added.

TERMINOLOGY.

The term ctenidium is used in the following pages to designate the entire respiratory apparatus of one side of the body. The halves of each ctenidium are called, in accordance with common though questionable usage, the gills, outer and inner respectively according to position adjacent to the mantle or to the body mass. Each gill, again, is composed of two lamellæ, called respectively the direct (or descending) and reflexed (or ascending). The same terms are employed in describing the limbs of the filaments composing the lamellæ. It will be noticed that the reflexed lamellæ (and filament limbs) of the outer and inner gills are turned in opposite directions — that of the inner gill toward the body, that of the outer gill toward the mantle. The *Mytilus* gill is of a very simple filibranchiate type in which the interfilamentary connections (between neighboring filament limbs of the same lamella)

[1] The studies upon which the present paper is based were carried on largely in the Marine Biological Laboratory, Woods Hole, Mass., and the Harpswell Laboratory, South Harpswell, Me. I am happy at this opportunity to express my appreciation of the courtesies and assistance extended by the directors of these institutions, Professors C. O. Whitman and J. S. Kingsley, and by their associates.

are represented solely by tufts of interlocking cilia, the ciliated disks (Fig. 7, *B*, *a*). The interlamellar connections are also very simple, consisting of hollow, cylindrical bridges, several of which extend between the two limbs of each adult filament (Fig. 7, *B*, *b*).

Résumé of Previous Investigations.

The classic on the development of the gill in the Lamellibranchiata is the paper by Lucaze-Duthiers ('56) entitled "Mémoire sur le développement des branchies des mollusques acéphales lamellibranches." A preliminary report ('54) preceded it. The first anlage of the ctenidium is described as a row of papillæ arising inside the mantle, along the line of juncture of mantle and body. These lengthen, bend back upon themselves, and become the filaments of the inner gill. Later a second series of similar papillæ form just outside the first, and develop similarly into the outer gill. Each gill thus passes through a stage in which it is composed of a row of unbent filaments hanging down in the mantle cavity.

The work of Lucaze-Duthiers was preceded by two interesting papers by Lovén ('49), in which young bivalves of several species are described and clearly figured with the ctenidium consisting of from three to ten free filaments belonging to the inner gill. The material was obtained from the plankton; and the identification (*Mya*, *Tellina*, *Mytilus*, *Mactra*, and *Nucula* are hesitatingly named) was recognized as very questionable by Lovén himself. Moreover, little account of the development is given.

Since the time of Lovén and Lacaze-Duthiers the investigation of gill development has been extended to a considerable number of widely separated genera of lamellibranchs.

Early stages of *Cyclas* were studied by O. Schmidt ('54); and his work on this genus has been extended by Leydig ('55), Stepanoff ('65), and Ziegler ('85), while Lankester ('75) gives brief notes on the closely allied genus *Pisidium*.

Unio and *Anodonta* have been described by Braun ('78), Schierholz ('78, '89), and F. Schmidt ('85).

The earliest stages of the gill of *Teredo* have been described briefly, and not very clearly, by Hatschek ('80). Sigerfoos ('96) has confirmed the observations of Hatschek and extended them to much later stages.

Ostrea has been briefly considered by Ryder ('84) and Stafford ('05).

Mytilus has been equally briefly described by Wilson ('87).

Dreissensia is known through the work of Korschelt ('91), Weltner ('91), and Meisenheimer ('01).

And, finally, the gill development of the peculiar and primitive Nuculidæ, *Nucula* and *Yoldia*, has been very carefully followed by Drew ('99, '01).

The accounts are for the most part brief, in some cases only incidental; and there are various points of divergence in detail. One such divergence appears at first to be fundamental. While Lacaze-Duthiers describes the first anlage of the gills as a series of isolated papillæ, the majority of the authors cited trace the ctenidium back to a longitudinal fold or ridge, which is secondarily constricted transversely and divided into the papillæ. Thus emphatically, among later writers, Ziegler for *Cyclas*, Schierholz for *Unio*, and Sigerfoos for *Teredo*. Drew describes a primary ridge and secondary papillæ in the Nuculidæ, but considers the change to be "due to unequal growth more than to constriction"; his figures, however, give evidence of considerable constriction.

On the other hand, Wilson confirms the early view of Lacaze-Duthiers for *Mytilus;* and my own observations are entirely in accord with this view. Concerning *Dreissensia* Korschelt ('91, p. 144) expresses himself thus cautiously: "Ob sie (the gills) in Form einer Falte angelegt werden, die sich schon sehr bald einkerbt und so jene vermeintlichen Papillen entstehen lässt, oder ob sie als wirklichen Papillen hervorsprossen, ist schwer zu entscheiden."

In the adult of all forms a continuous and undivided gill axis may be recognized, comparable with this embryonic fold or ridge. Upon this are carried the filaments, comparable with the embryonic papillæ. The whole divergence, then, reduces itself to a question of the relative time of development, and is one of minor importance. Much more significant is the general uniformity of development, and the recognition, by all the authors cited and in all the genera studied, of a stage in which each ctenidium is represented by a series of simple papillæ belonging to the inner gill; and of a later stage in which the outer gill is formed of a parallel series of corresponding papillæ.

Concerning the process of elongation and flexure by which the embryonic papillæ are metamorphosed into the definitive filaments of the adult gill in all forms except the very primitive Protobranchia the authors are again in the fullest agreement, so far as their attention has been turned to this later development; and this plan of development has been generally accepted as characteristic of the whole class.

Stages of Gill Development in Mytilus.

The following tabulation gives the size of the animal and the number of filaments present in the gills at certain of the more important stages in gill development. The figures can be taken only as approximate, as there is considerable individual variation; but they probably represent fairly accurate averages as they are selected from a much larger tabulation.

Stage.	Size.	Number of Filaments.	
		Inner Gill.	Outer Gill.
Earliest stage in which gills were observed.	0.30 mm.	3	0
Flexure of filaments of inner gill.	0.75 mm.	12	0
First appearance of outer gill.	1.40 mm.	20	(?)[1]
Flexure of filaments of outer gill.	1.60 mm.	25	15

Order of Succession of Gill Filaments In Mytilus.

Lacaze-Duthiers describes the order of the development of the filaments of the inner gill as from front to back, the most anterior being the first to develop; in the outer gill the development is described as extending in both directions, so that the first developed filaments would come to lie in the middle of the gill, with late developed filaments at both ends. My observations are in accord with those of Lacaze-Duthiers as regards the inner gill; but, in the case of the outer gill, amendment is required.

In very early stages, in which the filaments of the outer gill are mere papillæ, the longest papilla is not the most anterior, but is even posterior to the middle of the series. In one specimen with ten such papillæ, the seventh from the front is the

[1] Theoretically but a single filament should be present. No stage has been actually observed with less than three or four. Apparently several filaments appear almost simultaneously.

longest, the most developed, and, apparently, the oldest. Lacaze-Duthiers figures an almost exactly similar stage ('56, Fig. 6), as well as one a little older ('56, Fig. 7) in which the ninth of fifteen filaments is the longest. This suggests strongly a development of the gill in both directions in the early stages.

On the other hand, a comparison of these early stages with others much older shows that the position of the anterior end of the outer gill is somewhat definitely located relatively to the inner gill. There seems to be some individual variation; but the extension of the inner gill in front of the outer is measured in all cases observed by about ten filaments. Owing to the non-parallelism of the filaments of the two gills, it is very difficult to interpret figures that have not been drawn with reference to this particular point; but a study of Lacaze-Duthiers's plate seems to show ten such filaments in an earlier stage ('56, Fig. 6) and eleven in a later ('56, Fig. 7). As the inner gill is growing only at the posterior end, and as the filaments of the two gills are arranged somewhat definitely in corresponding pairs, this indicates almost certainly that the addition of filaments at the anterior end of the outer gill is confined to very early stages, if present at all. As regards these early stages my observations are very inconclusive.

In this connection it is also interesting to note that in a comparatively early stage, when the animal is only about 1.60 mm. in length and the outer gill is composed of only about sixteen filaments, the anterior filament of this gill is already strongly differentiated from its neighbors in both size and shape. It is decidedly thicker and longer, and is furnished with a very peculiarly enlarged and twisted termination. In both total preparations and sections it is very distinct from the other filaments and very easily identified. From this time on the development of the outer gill is clearly from front to back only, and the two gills develop *pari passu*.

Mode of Formation of Later Filaments in Mytilus.

The early filaments of the *Mytilus* gill follow the mode of development so beautifully worked out by Lacaze-Duthiers, and outlined briefly above; the later filaments follow a very different

mode. The change may be noticed in specimens but a little older than the latest stage figured by Lacaze-Duthiers. The ctenidium has grown beyond the rather small body mass and the posterior adductor, and extends free into the mantle chamber (Fig 1). The posterior part of the ctenidium is free from the mantle except for a light attachment due to the interlocking of

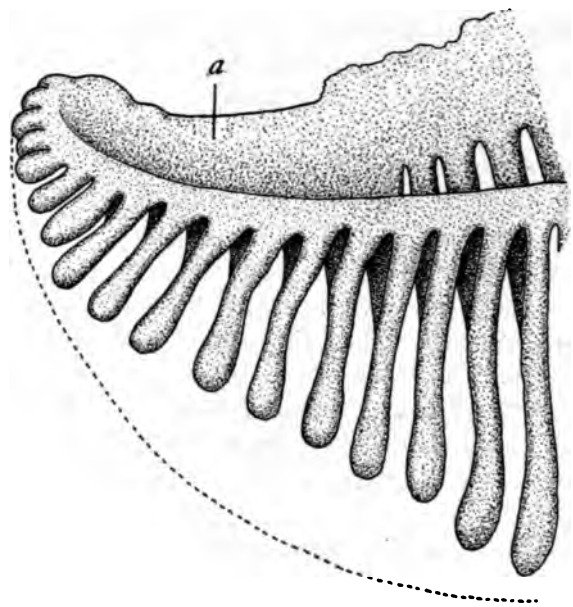

FIG. 1. Posterior tip of ctenidium of *Mytilus* of 2.5 mm. length, viewed from inner side. Outer gill omitted except for outline represented by dotted line. Magnification 170.

cilia. This suggests the connection of neighboring gill filaments (interfilamentary connections); but here there is merely diffuse ciliation and no specialized ciliated disks.

At the posterior end the gill axis (Fig. 1, *a*) is slightly curved upward; and around this curved end the developing filaments are closely crowded and assume a radial arrangement. The anterior filaments are attached to the ventral side of the axis and hang down ventrally; toward the end the position becomes more and more oblique, and then horizontal; while the youngest filament anlagen actually project upward from the dorsal side of the axis. Here all stages in filament development may be observed, ar-

ranged accurately in serial order. In Fig. 4 are seen isolated portions from the posterior end of the ctenidium. The specimens figured are selected from a large number of dissections, and are taken from several individuals of slighty varying size. Hence the discrepancy in the size of the figures, as all are equally magnified. All the stages figured have been observed again and again.

From the figures it is clearly seen that we are dealing with something very different from the comparatively long and slender

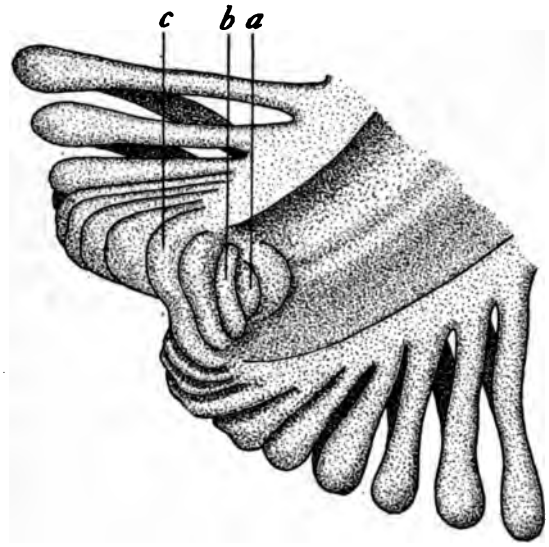

FIG. 2. Posterior tip of ctenidium of *Mytilus* of 3.0 mm. length, spread out, and viewed from dorsal side. Inner gill below and to right. Magnification 170.

rod-like papillæ observed in younger specimens. The youngest anlagen are rather transverse folds or ridges on the dorsal and posterior sides of the gill axis. At first these ridges are comparatively short and of uniformly convex contour (Figs. 4, *A*; 2, *a*; 3, *a*). Then the ridge elongates and becomes flat topped (Figs. 2, *b*; 3, *b*); and soon a process of differential growth or constriction leads to a notching of the ridge near its middle (Figs. 2, *c*; 3, *c*; 4, *B*). This notch divides the originally simple anlage into two slightly unequal parts, the larger belonging to the outer gill and the smaller to the inner. These two parts grow rapidly

(Figs. 4, *C*; 4, *D*), and may be followed readily into the characteristic filaments of their respective gills (Figs. 2 and 3).

In this connection it should be strongly emphasized that the filament anlagen here described are by no means the equivalent of the papillæ described by Lacaze-Duthiers, although their appearance in such a stage as that figured in Fig. 4, *D*, is strikingly similar. The undivided transverse fold (Fig. 4, *A*) contains potentially an entire filament of each gill; and each of its subdivisions (Fig. 4, *D*) is the equivalent of the two limbs of a filament placed side by side. On the other hand, the earlier type of anlage must be considered as the equivalent of only one limb of the definitive filament, or perhaps better, of the two placed end to end. In the earlier type the reflexed limb of the filament originates through a bending of the anlage; in the later type there is no bending, but rather a longitudinal splitting. The exact details of this splitting and the formation of the cavity within the gill, shown in Fig. 4, *E*, have not been worked out; but a study of a series of specimens such as those represented in Fig. 4 makes it clear that the process consists essentially in a thinning and ultimate perforation of the plate-like anlage of Fig. 4, *D*.

FIG. 3. Posterior tip of ctenidium of *Mytilus* of 4.5 mm. length, viewed from inner side. Magnification 170.

Corroborative evidence as to the nature of these later filament anlagen and their distinction from the earlier and more anterior ones is found in the character of the ciliation. A comparison of Figs. 4 and 5 is instructive in this connection. Fig. 5 is drawn from a section; but the filaments of the gills have been slightly reconstructed from neighboring sections, so that they are represented in their entirety. This represents an earlier filament of the outer gill (Fig. 5, *b*), with its characteristic rod-like form. Details of the ciliation have been omitted except for the some-

what diagrammatic representation of the highly specialized ciliated epithelium characteristic of the outer side of the filament, *i. e.*, the side turned away from the cavity of the gill. Thus in the inner gill (Fig. 5, *a*) the outside of each limb of the bent filament shows this characteristic ciliation; the papilla, or unbent filament, of the outer gill is ciliated on only one side, and that the side corresponding to the outside of the direct limb. In the later filaments (Fig. 4) the conditions are very different. In the

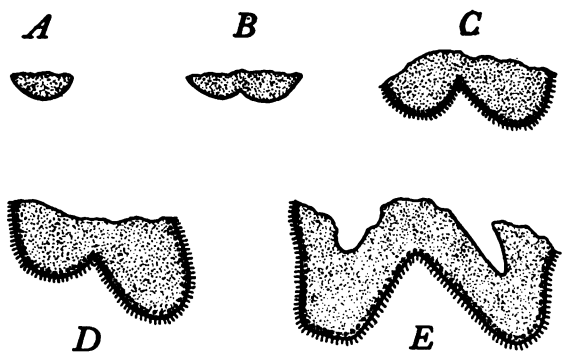

FIG. 4. Isolated filament anlagen from posterior tip of ctenidium of *Mytilus*. In all cases, anlage of outer gill at right. *A* and *B* from specimen of 2.25 mm. length; *C* from one of 2.00 mm.; *D* from one of 2.30 mm.; *E* from another specimen of 2.25 mm. Magnification 140.

two youngest stages represented (Figs. 4, *A*; 4, *B*) the tissue is distinctly embryonic with no differentiation; but in the three older stages (Figs. 4, *C*; 4, *D*; 4, *E*) the ciliated epithelium is clearly distinguishable, and is located on both sides of the anlage, showing the equivalence of the latter to both limbs of the flexed filament.

Another difference between early and late filament formation is found in the relations of the upper end of the reflexed filament limb. In the earlier filaments, the reflexed limb, developed at the free end of the direct limb, is primarily free. In *Mytilus* it retains this freedom;[1] but in many lamellibranchs the upper ends of reflexed filament limbs are secondarily fused with the

[1] Even in *Mytilus* there is a fusion of the upper ends of the reflexed filament limbs with one another to form a somewhat definite border to the reflexed lamella of the gill.

mantle (outer gill) or body (inner gill). In the later type of development the upper end of the reflexed limb is primarily united with the gill axis (Fig. 4, *D*), and only secondarily attains its freedom. The details of the process have not been studied. In the specimen represented in Fig. 4, *E*, this separation had not taken place, but the reflexed limbs were torn loose in the dissection as indicated by the rough edges.

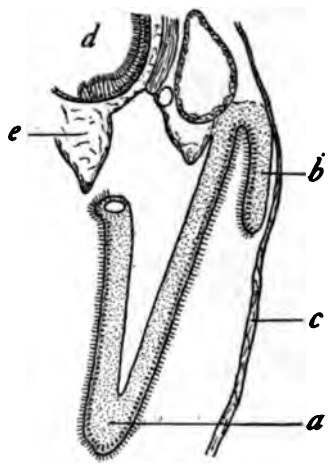

FIG. 5. Section through ctenidium of *Mytilus* of 1.6 mm. length. Section passes through posterior end of reduced foot. *a*, filament of inner gill; *b*, filament of outer gill; *c*, mantle; *d*, intestine; *e*, foot. Magnification 140.

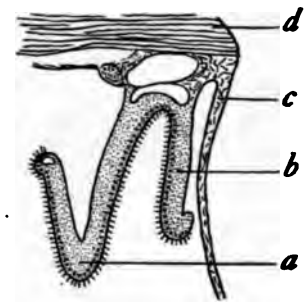

FIG. 6. Section through ctenidium of *Mytilus* of 1.6 mm. length. Section passes through middle of posterior adductor. *a*, filament of inner gill; *b*, filament of outer gill; *c*, mantle; *d*, posterior adductor. Magnification 140.

At first sight the two modes of filament formation seem fundamentally distinct; but the change from one to the other is bridged by all intermediate gradations. A comparison of Figs. 4, 5, and 6 gives some suggestion of the transition in the case of the inner gill; and the process is identical for the outer gill.

Figs. 5 and 6 are taken from the same animal, Fig. 6 being more posterior, thus showing a later filament in a younger stage of development. But it will be found by measurement that the ratio of the length of the reflexed limb to the direct limb of the filament of the inner gill is almost the same in the two cases, thus showing a precocious flexure of the posterior filament. A

further advance in this precocity leads to the condition found in a still later filament (Fig. 4), in which the flexure may be considered as having already occurred at the time when the filament is first budded forth.

Thus it is possible to conceive the later method of development as derived from the earlier; but it is equally possible, so far as mere geometrical relations are concerned, to reverse the series and consider the later mode as really primitive, the earlier mode as a specialization. It may be that the feeding requirements of the young animal are such as to require an adaptive modification in the direction of a speedy lengthening of the gill filaments; but it is likewise possible that the later mode of development is an adaptation to some factor or combination of factors in the life of the older animals. The startling similarity of the anlage of the later filaments to the gill in young specimens of the very primitive *Nucula*, as figured by Drew ('01, Fig. 46) make it very tempting to interpret the later type as primitive; but the structures are in such undeveloped condition that such comparisons are hazardous. The safe course is a suspension of judgment.

Filament Development in Other Lamellibranchs.

In connection with the above observations on *Mytilus*, the gill development in various other forms has been hastily studied for comparison. *Mya* is the only genus in which very young specimens have been observed. Here the early development seems to agree essentially with Lacaze-Duthier's description of *Mytilus*. Later stages have been studied in *Mya*, *Anomia*, *Modiola* and *Arca*. In all these genera the development of the later filaments follows the scheme here set forth for *Mytilus*. It is highly probable that this modification of development is characteristic of the later filaments in the Lamellibranchiata in general.

Development of Interlamellar Connections in Mytilus.

The contrast of the interlamellar connections in the closely related genera *Mytilus* and *Modiola* has been emphasized in a former paper ('98). In *Mytilus* these connections (Fig. 7, *B*, *b*) are irregularly cylindrical, and each contains a large blood cavity communicating with the cavities in the two limbs of the filament.

There are several such bridges between the limbs of each filament in a well-grown specimen. The particular filament figured had three interlamellar connections; but other filaments of the same animal showed four. In *Modiola*, on the other hand, the filament limbs are united by a continuous membrane (Fig. 7, *A*, *b*) which extends from the point of flexure for more than half the length of the reflexed limb. Sectioning shows that this membrane is composed of a very loose connective tissue with abundant blood spaces. The question immediately arises which of these types of connection is to be considered primitive. On other grounds than gill structure the view was expressed in a former paper ('98) that *Modiola* is the more primitive form. In that connection the *a priori* argument was advanced that the *Mytilus* type of gill filament might be easily derived from the *Modiola* type, while an independent origin of the interlamellar bridges of *Mytilus* would be more difficult to conceive.

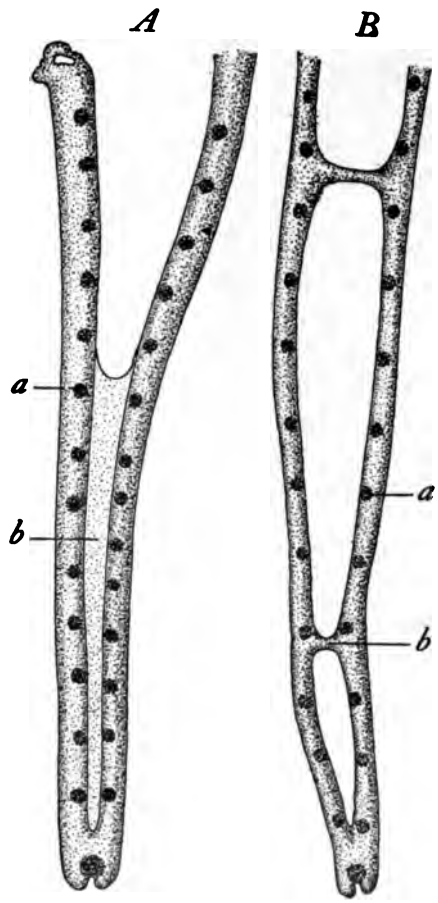

FIG. 7. Filaments of adult gill of (*A*) *Modiola* and (*B*) *Mytilus*. Only a part of *Mytilus* filament represented. *a*, interfilamentary connection (ciliated disk); *b*, interlamellar connection.

The examination of the development of these bridges confirms this view. Fig. 8 shows, in outline, the tips of five consecutive filaments of the gill of *Mytilus*. The filaments are arranged in the order of their occur-

rence in the gill; but the order of developmental stages is not the same. In the latest stage (Fig. 8, *E*) an interlamellar connection is present at a considerable distance from the tip of the filament; in the next younger stages (Figs. 8, *A*; 8, *C*) the connection is found nearer and nearer to the tip of the filament. Finally, in the earliest stage (Fig. 8, *B*), there is no distinct connection; but the distance through which the two limbs of the filament are in union at the tip as compared with the much shorter distance in Figs. 8, *A*; 8, *C*; or 8, *E*, shows a potential interlamellar connection in an undeveloped condition. The comparison with the

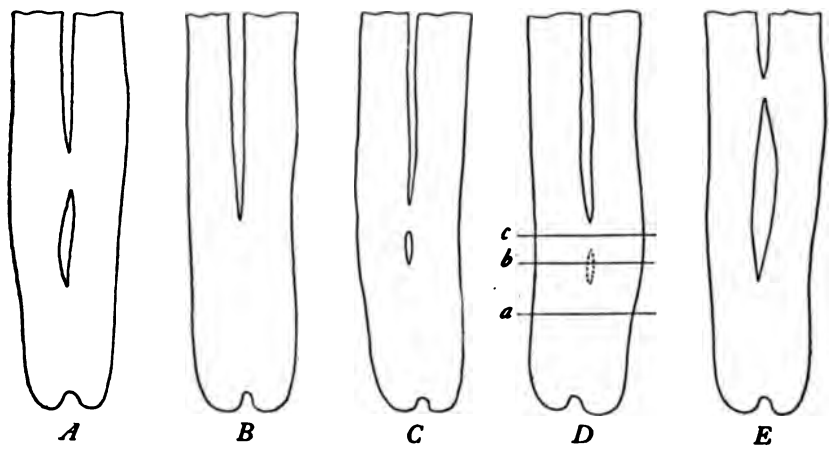

FIG. 8. Tips of five consecutive filaments of inner gill of *Mytilus* of 6 mm. length, showing formation of first interlamellar connection. Filaments represented in order of occurrence in gill; order of developmental stages of interlamellar connection is *B*, *D*, *C*, *A*, *E*. Magnification 110.

continuous connecting membrane of *Modiola* is evident, and it is hardly going too far to call this a "*Modiola* stage" in the development of the filament of *Mytilus*.

Perhaps the most interesting stage in the development of the interlamellar connection is shown in Fig. 8, *D*, which shows clearly the mode of formation of the *Mytilus* type of connection from its *Modiola*-like anlage. The filaments shown in Fig. 8 were first studied and sketched as total preparations, then sectioned. The study of the total preparation indicated that the area bounded by dotted lines in Fig. 8, *D*, was not an opening

like that separating off the interlamellar connection in the other filaments. The appearance was rather that of a thinner spot or constriction preliminary to complete perforation. Study of the sections fully confirmed this interpretation. In Fig. 9 three such sections are represented, their positions being represented by light horizontal lines in Fig. 8, *D*. The section nearest the tip (Fig. 9, *A*) shows a direct connection between the cavities of the two

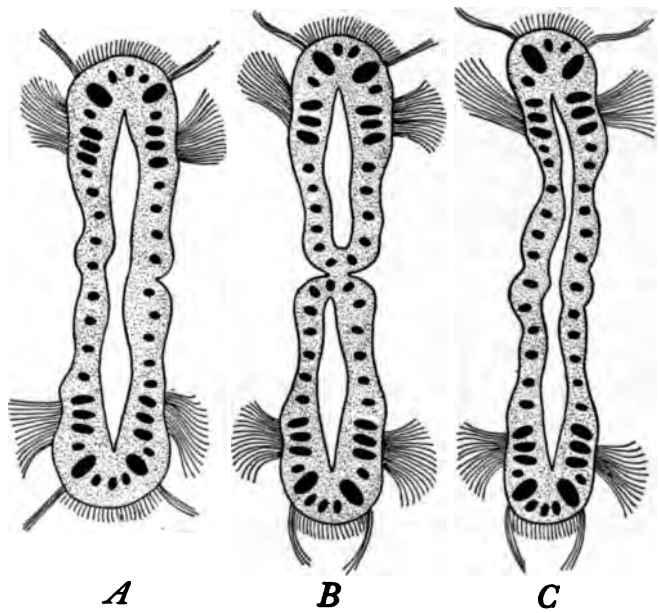

FIG. 9. Transverse sections of filament represented in Fig. 8, *D*. Position of sections shown by horizontal lines in Fig. 8, *D*, marked with corresponding letters. Magnification 540.

filament limbs in the neighborhood of the edge of the gill. Corresponding sections of other filaments show the same arrangement. The section farthest from the tip (Fig. 9, *C*) shows an essentially similar arrangement, which is duplicated by sections through the interlamellar connections of the other filaments. In the intermediate section (Fig. 9, *B*) the gill filament is strongly constricted, and the connection of the cavities of the two limbs is entirely interrupted. The next stage would be a complete perforation in place of this constriction, and the local separation of the filament limbs. Three successive degrees of such separation are shown in Figs. 8, *C*; 8, *A*; and 8, *E*.

Development of Interlamellar Connections in Other Lamellibranchs.

All stages in the development of the interlamellar connections in *Mytilus* have been repeatedly observed; but the investigation has not been extended to other genera. The similarity of the structure of the interlamellar connections in certain other forms, *e. g.*, *Astarte*, suggests similarity of development. But in the majority of the lamellibranchs the structure of these connections resembles *Modiola* rather than *Mytilus*.

General Conclusions.

There is a very striking parallel in *Mytilus* between the formation of the interlamellar connections (p. 71) and the separation of the two limbs of the later gill filaments (p. 68). In each case a flat, plate-like organ becomes locally thinner and is finally perforated. The parallel may be extended, though less fully, to the separation of the upper ends of the reflexed limbs of these later filaments from the gill axis (p. 70). As regards the two latter processes, at least, the parallel has been further extended to embrace several other widely divergent genera (p. 71), and is probably capable of general application. Moreover an essentially similar process may be recognized in the formation of slits in the primitive gill fold, as described by Hatschek ('80) and Sigerfoos ('96) for *Teredo*, and by Ziegler ('85) for *Cyclas*. The general process of perforation or separation in gill development appears to be of very common occurrence among the lamellibranchs.

The opposed process of fusion is equally characteristic. In the *Mytilus* gill this process is reduced to a minimum, but it may be noted in the fusion of the ends of the reflexed filament limbs. Other forms, however, show a high degree of fusion in the development of complicated inter-filamentary connections, represented in *Mytilus* by the simple ciliated disks; and an entirely different type of fusion has been noted in a former paper ('00) as occurring in certain of the complexly folded gills.[1] And, finally,

[1] This paper has been criticised by W. G. Ridewood ("On the Structure of the Gills of the Lamellibranchia," *Phil. Trans. Royal Soc. London*, Ser. 3, Vol. 195, 1903), who holds that the phenomena there described should be interpreted as the result of a splitting of filaments, not a fusion. I see no reason to change my previous view. As regards the present argument, splitting, like fusion, would indicate plasticity of the gill.

in many genera there is a more or less extensive fusion of the reflexed lamella of the outer gill with the mantle and of the inner gill with the body mass, or, posteriorly, with the corresponding lamella of the other ctenidium.

The frequency and prominence of these two opposed processes in development strengthen the conviction earlier expressed ('98) that the lamellibranch gill is an extremely plastic organ and one very liable to adaptive modification. This being the case, it is not an organ of fundamental phylogenetic importance, and has been given an altogether undue prominence in recent classifications of the Lamellibranchiata.

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THE EFFECT OF LOW TEMPERATURES ON HYDRA.

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In his experiments on *Hydra*, Greely, '03, observed that when the temperature is reduced, there occurs, what he termed, a reversal of vital phenomena. The body is quickly reduced to a resting stage, to an undifferentiated mass of protoplasm. He describes the changes as follows: "Whenever a *Hydra* is exposed to a temperature of 4° to 6° C., the tentacles gradually become shorter and thicker, and are finally completely absorbed into the body. As the absorption goes on, the ectoderm and endoderm cells of the tentacles lose their individuality and form an undifferentiated mass of protoplasm which is slowly taken into the body of the *Hydra*. The tentacleless body of the *Hydra* becomes slowly resolved into a dense spherical mass of coagulated protoplasm, in which no distinction between the individual cells can be made out, and remains in this condition as long as it is kept at a low temperature, but quickly forms tentacles and a double layer of cells again when it is returned to the temperature of the room. If *Hydra* in the earlier stages of the process of budding be placed at a temperature of 4° C., not only does the growth of the bud stop instantly but absorption of the bud into the body of the parent commences, and continues until all traces of the bud have disappeared. . . . Six or seven days are required for the complete disappearance of the bud. . . . Lowering the temperature brings about a reversal of vital phenomena and the formation of simple resting stages."

Greely, '01–'02, in an earlier series of experiments traced in protozoa a similar reversal of vital phenomena brought about by reducing the temperature. He found that when the temperature is lowered the protoplasm of unicellular forms coagulates, with accompanying loss of water and the cells pass into resting stages. His experiments on *Hydra* led him to conclude that similar changes may take place in metazoa.

Reversal of vital phenomena due to causes other than reduction of temperature has also been described. Loeb, '00, in campanularian hydroids produced similar effects in the polyps by bringing them in contact with solid bodies. In the same paper he describes like changes brought about by gravity. In *Antennularia* he found that when the branches are placed horizontally the polyps on the lower side are quickly absorbed.

Miss Thacher, '03, working on *Eudendrium*, *Pennaria*, and *Campanularia*, repeated Loeb's experiments, studying at the same time the histological changes. She found that the absorption as described by Loeb is not due to contact with solid bodies, but that it is a true degeneration caused by unfavorable conditions. At all times whether in contact with foreign bodies or not she found that the polyps of these hydroids are absorbed when brought into the laboratory and that the absorption is always preceded by degeneration.

Gast and Godlewski, '03, describe a similar degeneration of the polyps of *Pennaria* when kept in the laboratory.

The series of experiments described in this paper was begun with a view only of determining the histological changes which take place in *Hydra* when the temperature is lowered. Greely in his work considered merely the grosser structural changes. It was soon found that *Hydra* when subjected to low temperatures, often, in fact, usually do not behave as described by Greely. Exposure to a temperature of 2° C. for nine days, that is two degrees lower and three days longer than he found necessary to obtain a complete resting stage, if other conditions such as the composition of the water in which the *Hydra* are kept are unchanged. produces little or no effect on the structure. There may be no indication whatever of a reversal of vital phenomena. Because of this direct contradiction of results, it was thought advisable to repeat Greely's experiments, using as large a number of *Hydra* as possible.

Hydra fusca and *Hydra viridis* were the forms used. *Hydra fusca* is more favorable for experimental work than is *Hydra viridis*, since it is much more resistant to changes in the composition of the water; and also to sudden changes in the temperature. However with proper care either *Hydra* may be employed.

During the experiments the *Hydra* were usually kept in water from the ponds where they had been collected, although they appear to live as well in ordinary tap water. The water was changed frequently since even at low temperatures there is considerable evaporation.

When the *Hydra* were to be studied histologically they were fixed in an extended condition in a hot corrosive-acetic mixture, run up through the graded alcohols, imbedded in paraffin and cut in sections, 5μ thick. The sections were stained in haematoxylin-eosin.

The series includes about twenty experiments on something like seventy-five *Hydra*. From this number, five typical experiments have been chosen for description. Two of these were on *Hydra* collected in the winter, three on those collected in the summer.

Experiment 1. — February, 1907. One well-expanded brown *Hydra* was placed in an ice box, kept at a temperature of 2° C. The *Hydra* slowly contracted. At the end of nine days it was removed and appeared then as shown in Text-fig. 1, *B*. The body and tentacles had contracted until they were about one third the normal expanded length. The body had decreased somewhat in volume as if shrinkage had taken place. Upon removal from the ice box the temperature of the water around the *Hydra* was quickly raised to that of the room. When the temperature reached 8° C., the *Hydra* began to expand and by the time it had reached 10°, the *Hydra* had stretched to the length shown in Text-fig. 1, *C*. It had just the appearance of a normal expanded *Hydra* except that the tentacles were slightly shorter and somewhat opaque. When stimulated the *Hydra* rapidly contracted to the size shown in Text-fig. 1, *A*. Viewed under the low power of the microscope, while still alive it showed apparently normal structure: two distinct cell layers, nematocysts, etc. Ten minutes after its removal from the ice box the *Hydra* was fixed. Sections of the fixed *Hydra* showed practically normal cell structure, Fig. 6. The *Hydra* used in this experiment was taken from a pond having a temperature of about 10° C.

Experiment 2. — November, 1906. A brown *Hydra* with a small bud was kept at a temperature of 4° to 6° C. for eight days.

(The *Hydra* had been collected in a pond having a temperature of 12° C.) When removed from the ice box both body and tentacles were about one half contracted. The bud was about the same size it was when the experiment was begun. Sections showed body, tentacles and bud all with distinct cell structure, Figs. 1–4. The minute changes in structure shown by these sec-

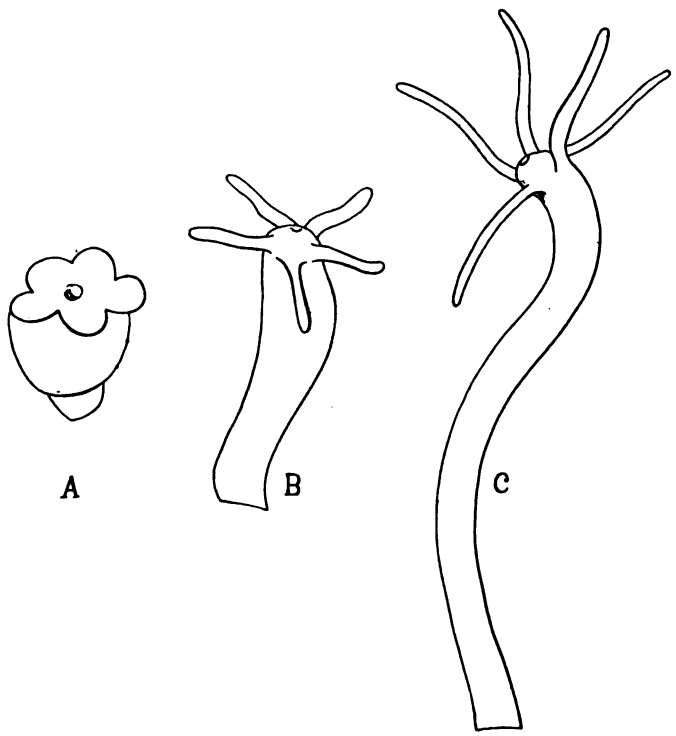

FIG. 1. *Hydra* after an exposure of nine days to a temperature of 2° C.; *B*, when taken from the ice box; *C*, two minutes later, the *Hydra* now at the temperature of the room; *A*, one minute later still; the expanded *Hydra* had been stimulated and rapidly contracted into this small mass.

tions will be described in detail in a separate paragraph farther on.

Experiment 3. — July, 1907. A brown *Hydra*, taken from a pond having a temperature of 30° C. was kept at 4° for six days. It gradually contracted and when removed appeared as drawn in Text-fig. 2, *A*. The body was a spherical mass with tentacles almost completely withdrawn. As the temperature was raised it

began to lengthen but could not stretch out to more than one third the length of the normal expanded *Hydra*. The *Hydra* was fixed and sectioned. The sections show distinct cells but in places, especially in the tips of the tentacles, there are marked cytological changes, changes such as might be brought about by a rapid loss of water.

Experiment 4. — July, 1907. The *Hydra* used in this experiment was collected at the same time as that used in experiment 3. It was a large brown *Hydra* with two buds, one large with long tentacles the other small with no tentacles. It was kept at a temperature of 4° to 6° C. for six days. At the end of the experiment it was firmly contracted, Text-fig. 2, *A*. When the temperature was raised it rapidly became active and in a few minutes extended until it appeared as shown in Text-fig. 2, *B*. It seemed incapable of expanding farther. The *Hydra* was fixed and sectioned. It still had clear cell structure, Fig. 5.

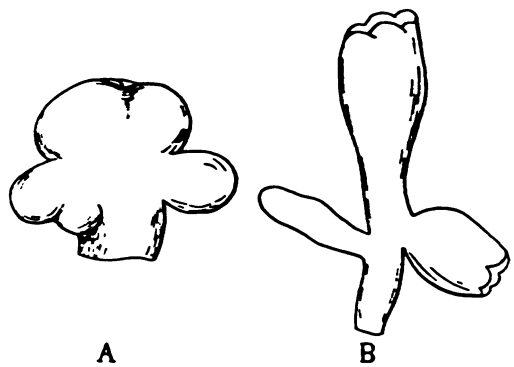

FIG. 2. A budding *Hydra* after an exposure of six days to a temperature of 4°; *A*, when taken from the ice box; *B*, two minutes later when brought to the temperature of the room.

Experiment 5. — July, 1907. A *Hydra* taken from a pond at a temperature of 30° C. was kept for seven days at 4°. At the end of the time it was contracted into an oval mass, the tentacles showing only as tiny knobs. When brought to the room temperature it could expand only very slightly, so little, in fact, that some magnification was necessary in order to detect it. When observed under the microscope while still alive no cell layers could be seen. Sections of the *Hydra* after fixation,

in only a few places showed cell boundaries. The body was in the main a mass of protoplasm with degenerating nuclei scattered through it, that is a syncytium. The nuclei were small and pycnotic, showing direct evidence of degeneration.

From the above experiments it will be seen that the changes which take place in *Hydra* when subjected to low temperatures, are somewhat variable. Those collected in summer, when the water in which they have been living is warm react differently from those collected in winter when the water is cold. In *Hydra* from cold ponds (temperature 8° to 12° C.) lowering the temperature to 2° and keeping it there for as long as two weeks has little effect except to cause contraction for the time being. As soon as the temperature is raised, they assume their ordinary expanded form. Usually the tentacles are slightly altered. They become opaque at the tips and cannot expand as far as before exposure to cold. *Hydra* that are budding show no absorption of the bud such as described by Greely. As soon as such *Hydra* are placed at room temperature the buds, as well as the parent body, become actively contractile.

Hydra collected in summer when the pond water is warm show more marked effects when the temperature is reduced. They contract into small masses and when brought, after several days exposure to cold, into water the temperature of the room, do not immediately expand to their normal length. Even in summer *Hydra* I have never observed absorption of the buds except in cases where there was distinct degeneration, due to some other condition than low temperature. Both summer and winter *Hydra*, when the temperature is reduced, lose in volume. This loss is more pronounced in the summer than in the winter *Hydra*.

The cytological changes brought about by low temperatures are interesting. They are in the main such as can be ascribed to loss of water. When more marked changes take place they are always accompanied by distinct evidences of degeneration. The temperature effects are always much more pronounced in *Hydra* collected in summer than those collected in winter. Fig. 6 is drawn from the endoderm of a winter *Hydra* which had been kept at a temperature of 2° C. for nine days. The structure is

almost identically that of a normal *Hydra*, except that the vacuoles in the cytoplasm are not quite so large and the nutrient spheres are not so numerous. The distended condition of the gland cells is worthy of note. This *Hydra* has practically the same structure throughout that one deprived entirely of food for an equal length of time would have. Here, then it is impossible to say whether there are any changes whatever due directly to reduction of temperature. In this *Hydra* there are, at any rate, no indications of Greely's reversal of vital phenomena. Figs. 1–4 are from sections of a winter *Hydra* after being kept eight days at a temperature of 4° to 6° C. Fig. 1 shows the entire thickness of the wall of the tentacle. The protoplasm of both endoderm and ectoderm cells is less vacuolated than in the normal *Hydra* and the nuclei, especially those of the interstitial cells are smaller and more deeply staining. In Fig. 2 the differentiation of the endoderm of the body into endoderm cells and gland cells is apparent. Even in the bud of this *Hydra* the cells are still intact. Fig. 4 shows a foot cell still filled with secretion. The sections of this *Hydra* show no changes that might not be directly due to loss of water.

In a *Hydra* collected in summer and placed at a temperature of 4° to 6° C. for six days the cytological changes are much more pronounced than in the two winter *Hydra* just described (see Fig. 5). Here the cells are much smaller than normal, the protoplasm is free from vacuoles, gland cells cannot be distinguished in the endoderm, and the nuclei of all the cells are very small and deeply staining. Many of the nuclei, especially those of the interstitial cells, look as if the nuclear sap had been almost entirely extracted leaving only the much condensed chromatin within. In a few places in this *Hydra*, most apparent at the tip of the tentacles, the cell boundaries are indistinct. Where such is the case the nuclei always are beginning to degenerate.

In *Hydra* like the one described in experiment 5, where, after exposure to cold, the body practically loses power of movement even after the temperature is raised, the cell structure is often entirely obliterated. The nuclei are in such cases always pycnotic and both nuclei and cytoplasm show every evidence of degeneration. Since it is seldom that *Hydra* are found which behave in

this manner when the temperature is lowered, it is probable that the loss of cell boundaries is due to some other condition than change of temperature. An entirely similar effect may be produced by keeping the *Hydra* in water that is not frequently renewed. It is possible that the reversal of vital phenomena described by Greely may have been merely a degeneration brought about by some such unfavorable condition. That he was able to get such *Hydra* to return to normal after bringing them to the room temperature for several days is no indication that they had been only in a resting condition. If after the temperature was raised the adverse conditions were at the same time removed, then any intact cells which may have escaped degeneration, might quickly regenerate the entire body. The ability of *Hydra* to regenerate from a few cells is well known.

Greely's statement that *Hydra* when kept at low temperature are resolved into undifferentiated protoplasm is misleading. It is impossible to tell whether he meant merely that the cell boundaries are destroyed, the nuclei remaining intact; or whether the nuclei too, are broken down so that the body is made up of a simple protoplasmic mass entirely devoid of differentiation. The former is probably what he meant. In such event it is possible that after all cell boundaries are destroyed regeneration may take place, but in this series of experiments no evidence of this has been seen. It is most likely that in all his *Hydra* that passed from his so-called resting stage to a normal condition, there had been only a partial destruction of cells, and that the few remaining cells regenerated the body.

Though Greely's results were not corroborated by these experiments yet his statement that reduction of temperature does bring about a loss of water is substantiated. All the effects due to lowering the temperature I think can be ascribed to this cause.

In concluding, it may be repeated that reduction of temperature for the length of time mentioned by Greely does not cause *Hydra* to be resolved into undifferentiated protoplasm. When this does take place it is due to unfavorable conditions and is a degeneration effect and not a temperature effect.

This work was done in the Zoölogical Laboratory of the University of Missouri under the direction of Professor Lefevre, to whom I am indebted for many valuable suggestions.

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EXPLANATION OF PLATE IV.

Abbreviations. *En*, endoderm cell; *ec*, ectoderm cell; *l*, supporting lamella; *ic*, interstitial cell; *nut*, nutrient sphere; *nem*, nematocyst; *s gl*, secretion granule; *gl*, gland cell of the endoderm; *p*, pigment; *cnid*, cnidoblast cell.

FIG. 1. Cross-section of the tentacle of a *Hydra* exposed eight days to a temperature of 4° to 6° C. *En*, endoderm; *nut*, nutrient sphere; *l*, supporting lamella; *ic*, interstitial cell; *ec*, ectoderm cell; *nem*, nematocyst.

FIG. 2. Endoderm from the body of the same *Hydra* as Fig. 1. *En*, endoderm cell; *gl*, gland cell; *p*, pigment; *nut*, nutrient sphere.

FIG. 3. Cross-section of the body of the bud of the same *Hydra* as Fig. 1. *En*, endoderm; *l*, supporting lamella; *ec*, ectoderm.

FIG. 4. Gland cell from the foot of the *Hydra* described in Fig. 1. *S g*, secretion granules.

FIG. 5. Cross-section of the body wall of a *Hydra* exposed for six days to a temperature of 4° to 6° C. This *Hydra* had been collected in summer from a warm pond. Notice the few vacuoles in the cytoplasm. *P*, pigment; *en*, endoderm; *l*, supporting lamella; *cnid*, cnidoblast cell; *nem*, nematocyst; *i c*, interstitial cells.

FIG. 6. Endoderm cells from the body wall of a *Hydra* which had been exposed to a temperature of 2° C. for nine days. The structure is almost identically that of a normal *Hydra*. *Nut*, nutrient sphere; *gl*, gland cell; *p*, pigment.

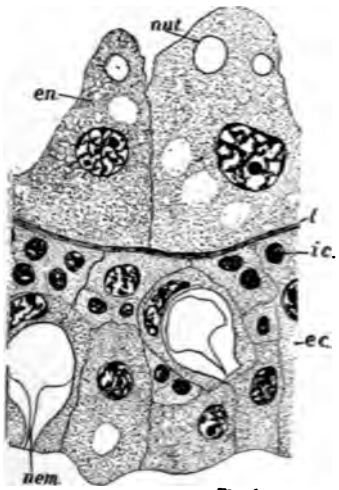

Fig. 1

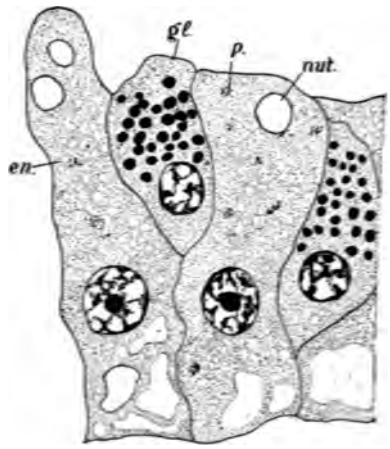

Fig. 2

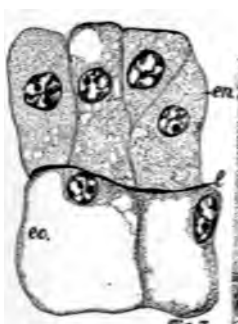

Fig. 3

Fig. 4

Fig. 5

Fig. 6

AMITOSIS IN THE MALPIGHIAN TUBULES OF THE WALKING-STICK (DIAPHEROMERA FEMORATA).

WM. S. MARSHALL.

Schindler[1] in his paper on the Malpighian tubules of insects describes both direct cell division and budding of the nucleus. In amitotic division he notes a regular form in which the nucleus is first cut in two and then followed by the cell (in *Sarcophaga carnaria*), and also, in *Lophyrus pini*, a peculiar method in which the division passes from one side of the nucleus towards the other.

While working on the anatomy of the walking-stick it was often noticed, in studying sections through different parts of the body, that mitotic figures were quite abundant. In looking over serial sections cut through the head, thorax, or abdomen, mitosis was often observed in cells in the fat, tracheæ, epithelial cells of the ovarian tubules, etc., but nothing of the kind was ever seen in the Malpighian tubules. Later, all the slides in which any Malpighian tubules could be found, were reëxamined in an endeavor to find dividing nuclei. Other slides were then prepared, both sections and entire tubules, etc., and, while no mitotic figures could be found, some nuclei dividing amitotically were seen.

Attention has already been called[2] to the two kinds of Malpighian tubules in *Diapheromera femorata;* these differ in their size and in their position within the body of the insect. In both kinds of tubules the cells are binucleate.

The greater part of the material used was taken from mature or nearly full grown insects. Tubules were also prepared from a number of walking-sticks about one third full grown. Besides these two stages a number of the insects were hatched out in the laboratory and the tubules taken from some which were not more than four to six days old. Embryos in which the tubules had developed were also sectioned. This gave four different stages;

[1] E. Schindler, "Beiträge zur Kenntniss der Malpigi'schen Gefässe der Insecten," *Zeit. wiss. Zool.*, Vol. XXX., 1878.

[2] W. S. Marshall and H. H. Severin, "Ueber die Anatomie der Gespenstheuschrecke, *Diapheromera femorata*," *Arch. Biontol.*, Vol. I., 1906.

embryos, specimens but a few days old, insects one third full grown and mature insects.

In a longitudinal section through an embryo two or three weeks before it was ready to emerge from the egg, the Malpighian tubules were easily seen and the condition of the nuclei noted. The tubules were narrow and short but, proportional to the length of the body of the insect, were, I judge, about the same as in mature specimens. In these tubules a few mitotic figures were seen (Fig. 1) but no instance of a direct nuclear division could be found. It was impossible to find any cell boundaries and nothing could, at this stage, be determined as to the binucleate character of the cells. In the tubules of mature insects the nuclei often lie in pairs, the two of each pair being nearer to each other than to the others; each pair being within a single cell. In the embryos studied the nuclei were very much crowded together and no such arrangement was possible; this crowding together of the nuclei made the relationship in size of nucleus to cell very different from what was found in the mature insect where the cell was, in proportion to the size of its nuclei, very much greater than in the embryo.

In the tubules of very young walking-sticks, four to six days old, no dividing nuclei were seen. Both in structure and in relative size proportional to the tubule, or rather that part one might imagine to be the cell, the nuclei were here similar to those found in the embryo. Here and there two nuclei were seen with their opposing surfaces very close to or touching each other — if cell boundaries had been visible these two nuclei would, no doubt, have been within the same cell. In these young insects it was noticed that other organs did not show nearly so many mitotic figures as were found in maturer specimens.

In insects about one third grown the cells of the tubules were, in proportion to the nuclei, much larger than in the younger insects. In the old specimens, none of which had completed egg-laying and some had not yet begun, no mitotic figures were ever seen although, as already mentioned, nuclei dividing indirectly were fairly abundant in the other organs of the body. Thousands of cells were examined from the older insects and no trace of mitosis was ever seen in the Malpighian tubules.

There is no visible difference in the nuclear division in the large and in the small tubules, and, while nuclei from both kinds are figured, a description of one will hold good for both. The size of the nuclei of the larger tubules is much greater than of the smaller ones. Each nucleus contains a number of chromatin granules, held in a reticulum, and several nucleoles which stain with the safranin of Flemming's triple stain and with the fuchsin of an acid-fuchsin-methyl green solution. The nucleoles are of different sizes and are scattered irregularly within the nucleus: in division some pass to each of the daughter nuclei. The size of the nuclei in each tubule varies considerably as does their position, some lying with their long axis across and some with it parallel or oblique to the tubule. Most of the cells are arranged with their long axis parallel to the length of the tubule but they do not all occupy this position. The nuclei generally divided parallel with the tubule although some oblique ones were seen.

At the commencement of division the nucleus first assumes an irregularly oval outline very similar to the resting nuclei except a somewhat greater elongation (Fig. 5). The nucleus then narrows transversely (Fig. 2) until it becomes apparent that it is dividing amitotically (Figs. 3 and 4).[1] An examination of nuclei at this stage shows that the nucleoli are about to become fairly evenly distributed between the two daughter nuclei; all those not near the center of the nucleus continue in or near their original position while those near the center are pushed, as this part grows narrower, into one or the other of the daughter nuclei. As the central part becomes more of a connecting strand the two main portions change their outline becoming more circular and losing the elongated appearance they had in the earlier stage. Just after the completion of division the daughter nuclei project in a point towards each other showing where the connecting strand has severed (Fig. 6).

While most of the cells are binucleate it was found that a number of cells contained but a single nucleus. All cases of amitosis seen were found occurring in the nucleus of the uninucleate cells. In the binucleate cells amitosis was never seen.

[1] J. B. Carnoy, "La Cytodiérèse chez les Arthropodes." *La Cellule*, Vol. I., 1885.

It would seem that in the Malpighian tubules of the embryo some of the cells were left with but a single nucleus and that later, by amitosis, these cells became binucleate.

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EXPLANATION OF PLATE V.

All figures drawn with a camera-lucida.

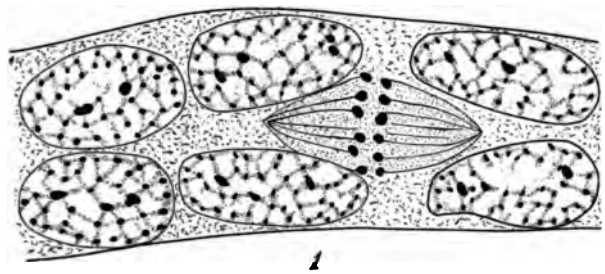
FIG. 1. Small piece of a Malpighian tubule from an embryo walking-stick. × 2,200.

FIG. 2. A nucleus showing early stage of amitotic division; from a large tubule. × 750.

FIGS. 3 and 4. Nuclei in a somewhat later stage; both from small tubules. × 1,200.

FIG. 5. Elongation of nucleus preparatory to division; from large tubule. × 1,200.

FIG. 6. Nucleus of large tubule after completed division. × 1,200.



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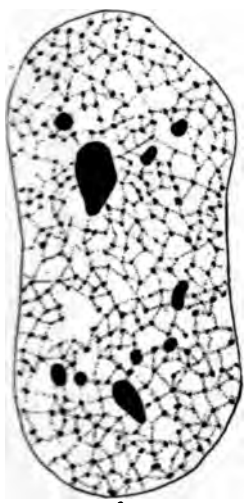

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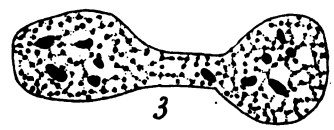

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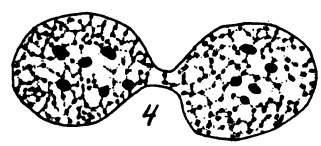

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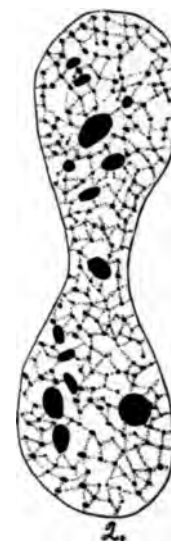

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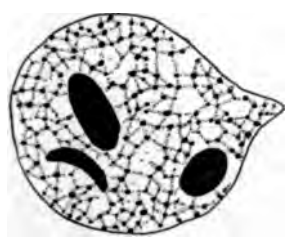

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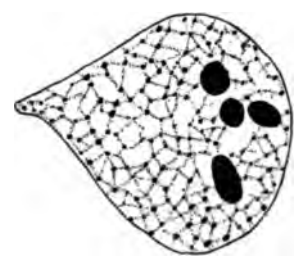


NOTES ON A FEW CŒLENTERATES OF WOODS HOLL.[1]

CHAS. W. HARGITT.

The following notes concerning a few Hydrozoa and other Cœlenterates, some new and others more or less rare, of the Woods Holl Region, and made chiefly during the summer of 1906, are published in the belief that portions of them at least are of more or less interest, particularly in view of the biological survey of the region now under way under the direction of the Bureau of Fisheries. It seems also worth while noting certain biological aspects of these organisms which are but indifferently recognized.

HYDROIDS.

EUDENDRIUM.—Among the prolific hydroid fauna of the region, few if any genera of Gymnoblastea are more abundantly represented both in species and individuals than *Eudendrium*. And among the species of *Eudendrium* perhaps none is so conspicuous in size or so abundant as *E. ramosum*. About docks, piers, in tide pools, in open waters and water of a depth of ten to twenty fathoms or more, the species is more or less abundant everywhere. Apparently the first record of the species in American waters was by McCrady in his now classic monograph ("The Gymnophthalmata of Charleston Harbor," p. 166), and by him identified with the *E. ramosum* of Europe.

It has long been a query in the mind of the present writer why, in the rather extended and painstaking work of Professor Agassiz, there seems to have been no acquaintance with this species. In his elaborate monographs on these forms he only mentions this species as a native of European waters. And in the later "Catalog of North American Acalephæ" of A. Agassiz (p. 160), it is only mentioned as having been taken by Clark in Charleston Harbor. And even as late as Verrill's "Invertebate Animals of Vineyard Sound" (1871, pp. 408, 734), its occurrence is merely mentioned, with no account of its characteristics or

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

distribution. Can it be possible that this species is a recent comer into this region, or has it become more prolific and abundant of recent years, or was its earlier presence simply overlooked by naturalists? So far as I know these queries must remain such, at least for the present. In this connection I desire to note the variable character of the species when found in deeper waters. As known in shallower habitats it is usually stout, with dense stems much fascicled and profusely branching; while from depths of fifteen or more fathoms it is often more slender, stems weak, colony less massive and complex, and with gonads of variable colors and characters, the whole resembling somewhat *E. dispar*.

EUDENDRIUM DISPAR. — This species most resembles the preceding in its general size and aspects of any of the series. While not to be ranked as especially rare, it is yet far from common. This may be due, in part at least, to the fact that its habitat is usually the deeper waters, ten to thirty fathoms, hence only available by means of the dredge. But this does not fully explain its comparative rarity, for the writer has collected by all known means for fifteen years throughout the region, and this species has not been taken on an average once per year during this period. It may therefore be ranked at present as rather rare.

In this note it is desired to call attention to the fact of the considerable variation in morphological characters shown by the species as taken from varying depths and other environmental differences of habitat. Agassiz in the original description of the species (*Cont. Nat. Hist.*, Vol. IV., p. 286), states that the branches and pedicels are extensively annulated. Later students have generally followed Agassiz in this respect. As a rule such is true in a majority of cases, but it should be pointed out that specimens are found in which there is considerable variation, the annulation resembling much more nearly that of *E. ramosum*. Again other specimens will be found in which the annulation extends to the entire stem, as well as to the branches and pedicels. The most distinctive difference, and most constant, is to be found in the character and position of the gonophores. These I have found to be very constant, and agree quite closely with the figures of Agassiz.

In distribution the species seems to be more common in Buz-

zards Bay though I have taken it several times in the deeper waters in the region of Gay Head.

EUDENDRIUM ALBUM.— This species was first described by Nutting in 1898. In a "Synopsis of the Hydroids" (*Am. Nat.*, Vol. XXXV., p. 310), I expressed some doubt as to the distinctness of Nutting's species. During the present year I have taken two colonies of this little hydroid, which seemed to differ in important points from *E. capillare* and *E. tenue*, the species which I had considered probably identical. In comparing these several species, having obtained specimens of *E. capillare* from Naples, there seems no longer any good reasons for doubting their distinctness.

EUDENDRIUM CARNEUM.— This species, first described by Clarke, was taken at three different points during the summer, namely, from piles of the docks of the Vineyard Haven Yacht Club; from fucus off Naushon; and later dredged off Gay Head. It is not a common species at Woods Holl. In general characters it might easily be mistaken for young colonies of *E. ramosum*, but may be distinguished usually in the sexually mature stage by its smaller size, rarely exceeding two inches in height and by the flesh red color.

HYDRACTINIA.— Two points of some interest will be noted concerning the local species of *Hydractinia*, first, the rather interesting range of habitat which characterizes it; and second, some facts bearing on the question of the affinities of the species.

As is well known the more familiar habitat of the species is the shell inhabited by hermit crabs. So general is this conception on the part of zoölogists that it is often given as *the distinctive* habitat, and that because of this peculiarity it is cited as one of the more common illustrations of *symbiosis*, and not infrequently pains are taken to show just wherein these creatures sustain essential relations of mutual helpfulness and interdependence. But every careful student of hydroids knows very well that *Hydractinia* has a range of habitat which would be clearly incompatible with the foregoing conception of necessary symbiosis. Agassiz long ago pointed out in his original description of the species, *H. polyclina*, that it was to be found covering rocks in

tide-pools, sometimes to the extent of several square feet. The same fact has also been pointed out by McCrady, Leidy, and others. During the current season I have records of the following modes of life: The general occurrence on shells occupied by hermit crabs; the maxillipeds of the lobster; the chela of the common crab, *Cancer irroratus;* stems of common rock-weed; dredged in Vineyard Sound on bit of waterlogged oak timber; finally in immense masses from piles of docks at Vineyard Haven, and still later from the carapace and legs of *Limulus.* The occurrence on the appendages of the crab and lobster are sufficiently similar to that on the shell of the hermit crab to make it devoid of special significance. But the occurrence and distribution in the other cases are certainly not compatible with any necessary commensal relations. On the other hand they go to confirm the suggestion made above, and also long ago suggested by Agassiz, that there is probably no essential advantage to this hydroid in its habitat on the shell of the crab. Certainly in the enormous colonies of the hydroid on these stationary substrata we cannot perceive any adverse conditions so far as the animals are concerned, for not only were the vegetative conditions among the most remarkable known, as shown by the enormous colonies, but they were apparently in the height of sexual development, both male and female colonies being abundant, and ladened with gonads.

The second feature to be noted, as intimated above, is the question as to the specific distinctness of local species. As is well known, Agassiz regarded it as specifically distinct from the European *H. echinata*, and designated the species as *H. polyclina.* In my Synopsis (*op. cit.*), I followed Allman in his rather emphatic doubt on this point, and designated the species as *H. echinata.* In connection with the unusual numbers taken during the present season, and their range of habit, I took occasion to go carefully over the subject once more, reviewing as carefully as possible all the evidence available, and find myself unable to distinguish any good grounds for regarding these two species, so-called, as sufficiently different to warrant the distinction. And when one recalls the fact that two systematists of the acknowledged renown of McCrady and Leidy both regard our species as

identical with the European, it should call for very convincing evidence to decide to the contrary. I do not overlook the fact that Professor Nutting, who has seen the European species, believes that Agassiz's early decision was correct, but the only evidence he cites in its favor is the "larger hydranths and less number of tentacles," of the English species, both of which characters are exceedingly variable ones, and hardly admissible alone from which to predicate specific distinction.

In some of the colonies collected during the summer I thought for a time that some such differences as Nutting indicated were present, particularly on those taken from piles. But upon a comparison of other colonies they were entirely lacking; and still other comparisons showed such apparently indistinguishable intergradations of characters that I was compelled to regard them as too variable to warrant the establishment of an independent species. I must therefore return to my earlier impression with increasing conviction, namely, that our species of *Hydractinia* is to be regarded as identical with that of European waters, and is therefore *H. echinata* Fleming, and not *H. polyclina* Agassiz.

CORDYLOPHORA. — This hydroid is known to have a very wide range of distribution, but so far as I recall at this time only a single species is known, namely, *C. lacustris* Allman. It was first found at Woods Holl by Professor Morrill in 1899, in Nobska pond, who kindly turned it over to me for identification.

Since then I have taken it in several similar ponds in the region and near Falmouth, and in fresh or brackish ponds on Marthas Vineyard. Indeed the hydroid seems to be quite generally distributed throughout the region. The more common habitat is on fragments of rock, or on bits of submerged sticks, eel grass, etc. Occasionally it forms large and rather complex colonies, the hydrorhiza forming an intricate network from which branching stems arise to a height of about an inch or slightly less. The reproductive season seems to be chiefly in spring or early summer, and sparingly in July. Colonies have been found later, September or October, but with no signs of gonophores. As I have pointed out in an earlier paper (*Zoöl. Bull.*, Vol. I., p. 205), it lives well under the artificial conditions of aquaria, but only

in its vegetative phases, no sexual organs appearing. Furthermore, if these be present when brought to the aquarium they soon show signs of degeneration, and later disappear.

CORYNITIS. — This genus was instituted by McCrady for a hydroid and medusa described by him from Charleston Harbor (*Proc. Elliott Soc. Nat. Hist.*, Vol. I., p. 131), and named in honor of Professor Agassiz *Corynitis agassizii*. Notwithstanding the fairly full description, especially of the medusa, the latter illustrated by good figures, a most remarkable confusion has crept into the literature in reference to the supposed affinities of the species. From material which has come into my possession within recent years, and from facts gathered therefrom it now seems possible to clear up the matter once for all.

About the time that McCrady described the above named species Agassiz also described a new hydroid which he designated as *Halocharis spiralis* (*Cont. Nat. Hist.*, Vol. IV., p. 239). For some unaccountable reason he subsequently came to regard this species as identical with McCrady's *Corynitis*, and on page 340 gives priority to the latter name, ranking *Halocharis* as a synonym. That this was not simply a clerical error is evident in that on page 344 he recognizes McCrady's *Zanclea gemmosa* as quite distinct from *Halocharis*, and this error is perpetuated by A. Agassiz in his "Catalog of N. Am. Acalephæ," p. 183. These errors have continued throughout the literature up to the present time, though as will be shown, it has later been determined that the medusa which McCrady described as *Zanclea*, or rather *Gemmaria gemmosa*, is liberated from a hydroid resembling Agassiz's *Halocharis spiralis*. That Murbach, who first observed the liberation of this medusa, was correct in identifying it with McCrady's *Gemmaria gemmosa*, I have abundantly satisfied myself at various times since. But he is clearly in error in attempting to identify it with McCrady's *Corynitis*, due no doubt, to the earlier error of Agassiz as already pointed out. Murbach is also in error in attempting to distinguish a generic difference between Agassiz's *Halocharis* and the *Gemmaria* of European writers, as I have elsewhere pointed out (*Mitt. Zool. Sta. Neapel*, Vol. XVI., pp. 574–577, "Medusæ of Woods Holl," 1904, p. 42).

In the summer of 1904 the writer described an apparently new species of hydroid from Long Island Sound, namely, *Syncoryne linvillei* (BIOL. BULL., Vol. VII., p. 351). Not having access at the time to McCrady's monograph, and with the current confusion above referred to still more or less dominant, the details of McCrady's description of *Corynitis* were wholly overlooked. A more recent and critical examination of this has clearly convinced me that the hydroid in question is quite identical in its generic relations with *Corynitis*, and should be so ranked hereafter. Whether it is specifically the same as *C. agassizii* must remain more or less uncertain, at least till it may be possible to have specimens of free medusæ for comparison, these having been lacking in the material from which my description was drawn.

FIG. 1.

That there may be no doubt whatsoever as to the facts concerned, or of the confusion alluded to, it seems worth while to present several of McCrady's figures with others, and to include also some summary of his descriptions.

Concerning the *hydroid*, his description is rather inadequate and indefinite. "The larva is a coryne with a short, thick polyp and few tentacula. The medusa-buds borne in the usual position, and the peculiar character of the tentaculiferous bell-margin is conspicuous at an early age." Of the habitat of the hydroid he says: "The coryne which bears this medusa is rather rare, as is also the medusa. It is found growing on sponges a little above dead low water mark. It has been found during the summer months and whether or not it exists during the winter

(as in all probability it does), has not been ascertained. A young bitentaculate, but free medusa, has been found as early as the fifth of June. A fully developed specimen has occurred in the end of July, while as late as the twelfth of September, buds were still produced from the coryne, Figs. 6, 7 and 8 having been drawn at this date. This leads me to say that I have not seen the actual separation of a bud from the hydroid, and its assumption of the form of Fig. 5. My confidence that they are one and

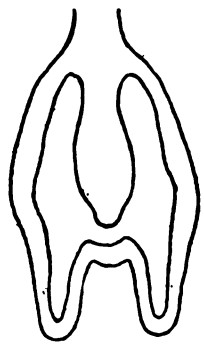


FIG. 2.

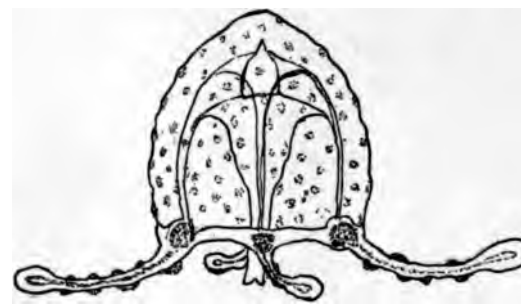


FIG. 4. *Corynitis agassizii.* (McCrady.)

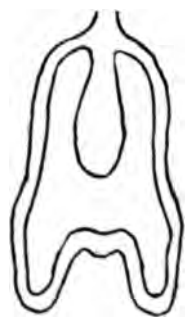


FIG. 3. *Corynitis.* (McGrady.)

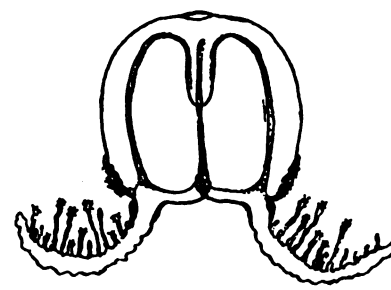


FIG. 5. *Gemmaria gemmosa.* (After McCrady.)

the same is due to the very marked and almost unmistakable peculiarities of the medusa, which are fairly exhibited in the buds while attached to their hydra."

As will be seen, there is little here from which one might attempt to identify the hydroid. Aside from the fact that it is designated as a corynid, and that it has a short thick hydranth with

few tentacles, the medusa-buds in the "usual position," no morphological features are given. The habitat and association with the sponge are interesting facts, but without taxonomic significance.

It is unnecessary to repeat here the characters by which *Syncoryne linvillei* is distinguished. A glance at the figure (1) of a portion of a colony, with the reference already cited, will suffice to afford ample opportunity for comparison to those concerned. I may add, however, as was intimated in the original description, that while having generic characters in many points conformable with those of *Syncoryne*, still there are points of considerable difference. And with McCrady's figures and description before one it is at once apparent that my species belongs to *Corynitis* and not to *Syncoryne*, as already intimated.

A comparison of the several figures of *Corynitis* and *Gemmaria* will make more evident the points under consideration. For example, a comparison of Figs. 2 and 3 which represent corresponding stages in the medusæ of *Syncoryne linvillei* and *Corynitis agassizii* will strongly suggest their close resemblances and probable generic identity, as already indicated.

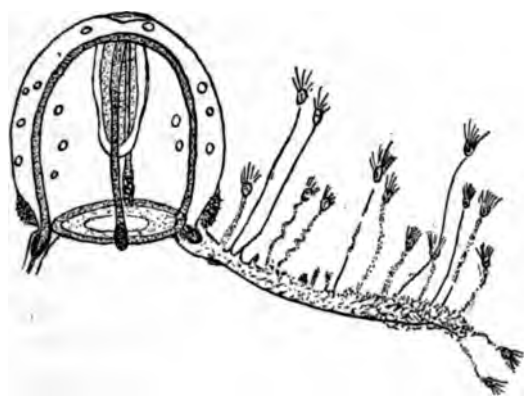


FIG. 6. *Corynitis agassizii.* (After Murbach.)

Futhermore, a comparison of Fig. 4, representing the free medusa of *Corynitis agassizii*, taken directly from McCrady's drawing, with Figs. 5, 6 and 7, representing medusæ of *Gemmaria*, will also show one at a glance the unmistakable distinctness of the medusæ portrayed. Fig. 5, copied from McCrady, represents

his well-known genus *Gemmaria*. Fig. 6, copied from Murbach's figure of *Corynitis*, confused by him with the very different genus of this name, gives a good picture of the medusa of *Gemmaria gemmosa*, to be discussed in the following section. Likewise, Fig. 7, from the writer's description of *Gemmaria implexa*, also shows beyond doubt the distinctive gemmarian features.

With these figures, and facts cited bearing upon the problems concerned, there can hardly be reasonable doubt as to the conclusions to be drawn. *Syncoryne linvillei* must be identified with the genus *Corynitis;* and the *Corynitis* of later literature must be identified with the genus *Gemmaria* of McCrady.

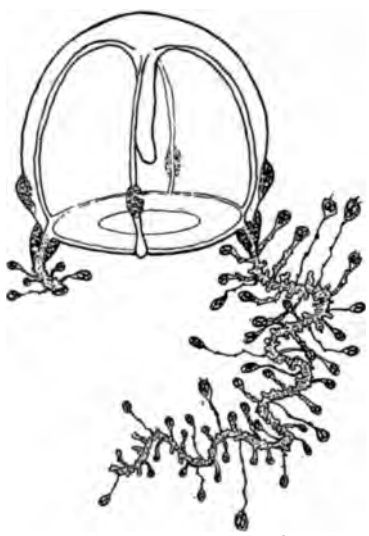


FIG. 7. *Gemmaria implexa.* (Naples.)

It is therefore to be hoped that the long-standing confusion here referred to may be once for all removed by recognition of the facts as given. And while it is too much to anticipate that similar confusion along kindred lines may be avoided entirely, the lessons of past experience should count for something in rendering their occurrence less frequent, or of such persistence.

GEMMARIA. — Attention has been directed to the confusion of McCrady's *Gemmaria* with his very different genus *Corynitis*. A similar confusion has also been more or less current as to the relations of *Gemmaria* and *Zanclea*. The latter was instituted by Gegenbaur for a medusa found at Messina, and recognized by McCrady as having certain points of similarity to his doubtful *Gemmaria*, which he believed however, to be quite generically distinct from *Zanclea*.

Having taken a medusa at Woods Holl during several years which is now well known as identical with McCrady's *Gemmaria*, and having kept these medusæ at various times and for considerable periods in the laboratory, I am convinced that they are beyond

doubt generically different from *Zanclea*. Briefly diagnosed, *Zanclea* was described by Gegenbaur as having four short oral lobes, four radial canals and the same number of marginal tentacles, the latter with numerous secondary appendages (Anhängen).

In at least two aspects there are important differences in *Gemmaria*, viz., the mouth is not marked by any distinguishable lobes or lips ; and second, there are only two marginal tentacles. These latter do not increase in number with age, so far as one may judge by having them long under observation. This is likewise true of *Gemmaria implexa*, taken by the writer at Naples, and described briefly in a paper in the Naples Mittheilungen (Bd. XVI., p. 574).

It would seem therefore that the genus *Gemmaria* of McCrady must be recognized as founded on thoroughly good characters, and that is it quite distinct from *Zanclea* of Gegenbaur. Hence we must also accept McCrady's *G. gemmosa* as a distinct species, and this name must entirely supplant that of *Corynitis agassizii*, as pointed out in the preceding section.

Colonies of the hydroid are quite frequently taken at Woods Holl, and from a considerable variety of habitats, *e. g.*, on shells of *Mytilis*, *Pecten*, serpulid tubes, pebbles dredged from various depths, pieces of waterlogged wood likewise dredged from similar depths, and from floating *Sargassum*. In one particular, however, there has been an interesting uniformity in every case which has come to my notice, namely, the colonies are invariably associated with encrusting polyzoa, usually *Schizoporella* or *Membranipora*, and almost always with those colonies characterized by pinkish or orange colored pigment, affording a background which resembles very remarkably the color of the hydroid. The hydrorhiza forms an intricate network over the polyzoön, but so far as I have been able to perceive there is no special evidence of mutualism between the organisms.

The following note concerning the peculiar nettling organs of the medusæ may not be amiss. The extension of the stalk bearing the nettling organ is apparently brought about by a sort of rotary, or oscillatory motion of the capsule, involving a spinning-like operation by which the thread becomes extremely delicate almost to the point of invisibility. After attaining its full extension the capsule continues its motion for a variable time,

when the stem finally contracts in a way to suggest that of *Vorticella*, though without the coiling of the stem as in the latter organism. I am inclined to regard the organ as probably possessed of a tactile function.

ECTOPLEURA. — While dredging off Gay Head on board the "Fish Hawk," July 15, 1907, I was fortunate in discovering on the carapace of a small specimen of the common spider crab, *Libinia*, among other hydroids not uncommon on this creature, including a species of *Campanularia* and *Halecium articulosum*, a small tubularian-like hydroid having a very short stem, large hydranth, the latter being crowded with racemose clusters of medusa-buds, some almost ready to be liberated. In appearance the hydranth, in its size and general aspects, was much like *Tubularia crocea*, though rather larger, and the oral tentacles fewer in number. A closer inspection under a lens, and later under the low power of the microscope, soon made it evident

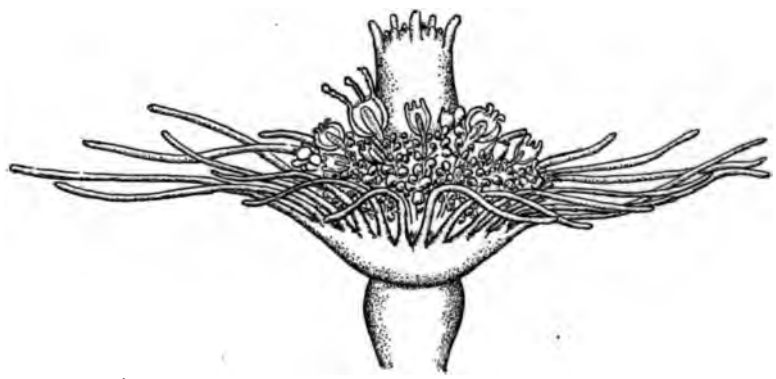


FIG. 8. *Ectopleura prolifica.*

that the specimen did not belong to *Tubularia*, and the liberation soon after coming to the laboratory of several medusæ made this doubly certain. An examination of the medusa soon showed that we had under examination a species of *Ectopleura*. Since no description of the hydroid of this genus has been recorded from this region it seemed worth while to have a careful sketch made of it, which is shown in Fig. 8.

The following are the chief points of diagnostic importance. Stem of hydroid very short, hardly exceeding 5 or 6 mm., and

devoid of definite perisarc. (This may be due in part to the habitat, on the crab, these animals being given to decorating themselves with various living organisms, such as hydroids, polyzoa, algæ, etc., and if this hydroid were there by such process its very short stem devoid of perisarc may be due to its recent transplantation.) The hydranth is rather large, with two whorls of tentacles, the basal series about twenty-four in number, long, and filamentous, much as in *Tubularia;* oral tentacles few and short, about ten or twelve, apparently in two series, one *very* short, merely bud-like. Hypostome of hydranth rather cylindrical, entire hydranth low vasiform, and with definite constriction below the body. Medusa-buds borne on body of hydranth in series of racemose clusters, as shown in Fig. 9. Growth of medusæ apparently rapid, the older forming the terminal portion of the cluster Color of hydranth pinkish red, tentacles paler.

FIG. 9.

MEDUSA. — Bell transparent, subspherical or when older oblately spheroidal; diameter when first liberated about .5 to .6 mm., and becoming but little larger after several days in the laboratory, Walls of bell rather delicate, though not flabby; velum very delicate, with tendency to evert during contraction. Radial canals four, rather open showing free movement of circulating fluid. Tentacles four, rather short, and terminating in knob-like masses of nematocysts, body of tentacles with nematocysts in scattered clusters of four to six in number usually, though some specimens seemed all but devoid of them. The entire exumbrella of newly-born medusæ is more or less dotted with clusters of nematocysts. A characteristic feature of the medusa is the presence of eight rows of nematocysts arching over the exumbrella from the basal bulb of each tentacle, a row on either side. Basal bulbs rather prominent, and with scattered granules of pigment throughout their substance. Manubrium relatively large or thick and not extending at any time to the level of the velum, mouth devoid of lobes or lips of any sort.

Color. — Bell very transparent, or with only the slightest tint of color by reflected light; tentacular bulbs and base of manubrium brownish red due to the presence of scattered pigment granules in the entoderm; tip of manubrium slightly bluish by reflected light. While the colors may be easily distinguished they are incomparably inferior to those of *E. ochracea*, a very nearly related species.

Dr. A. G. Mayer has informed me that the hydroid of *E. ochracea* has been taken by him at Newport and that it has much in common with the hydroid here described. The characters of the medusa are, however, very different from that of the former.

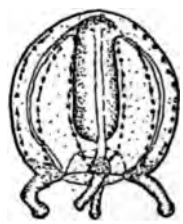


FIG. 10. Aspect of newly born medusa.

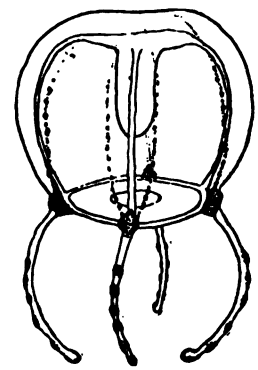


FIG. 11. Aspect of fully developed medusa.

Having taken those medusæ by hundreds for many years one could hardly confuse them with any other known species. It had seemed possible that this might be identical with McCrady's *Sarsia turricula* but a careful comparison of the original figures and description makes it perfectly certain that the species are wholly different; indeed I am inclined to believe that *S. turricula* is evidently of a different genus, as McCrady has indicated, and that Agassiz was in error in placing it under his newly established genus, *Ectopleura*. The only species with which the present one is at all comparable is the *Tubularia dumortierii* of Van Beneden, but here again there are points of difference, especially in the size and features of the medusa. It seems therefore rather probable that we have here a new species, or in any case a variety

entitled to identity, hence I am disposed to suggest for it the name *E. prolifica*, which may be specific or varietal, as the results of later comparison of material may warrant.

AGLAOPHENIA. — During the summer I collected from masses of *Sargassum* which was blown into the harbor from Vineyard Sound, a species which seems in all essentials identical with Fewkes' *A. minuta*. The hydroids were entirely devoid of gonangia, as was the case with the species as originally described by Fewkes, whose account was extremely brief, and without figures — "Found growing in great abundance on an Alga over the fronds of which the *hydrorhiza* extends." (*Bull. Mus. Comp. Zoöl.*, Vol. VIII., p. 132.)

Nutting has since described and figured the gonangia, using material collected by Fewkes, and indicated as types, though as already mentioned, the original description made no mention of the gonosome.

In a recent paper on "Bermuda Hydroids" Mr. E. D. Congdon has a brief note on the species, found there on *Sargassum*, but as in my specimens, devoid of gonangia.

So far as I am aware, the present is the first record of the species at Woods Holl, and this mention of it seems therefore important.

OBELIA. — The present note refers only to a single species, and of that only to the medusa, which up to the present time, seems to have been entirely unknown, or at any rate undescribed.

The material was collected by Mr. Geo. M. Gray at Woods Holl in April, 1906, and in the aquarium numerous medusæ were liberated. The hydroid was clearly *O. flabellata* Hincks.

The following synonymy is given by Haeckel, "Das Syst. d. Medusen," p. 177:

Thaumantias plana Sars., 1835.
Campanularia flabellata Hincks, Ann. Mag. Nat. Hist., 3d, Vol. XVIII., p. 297.
Obelia flabellata Hincks, Brit. Hydr. Zoöphytes, p. 157.
Eucope plana Agassiz, Cont. Nat. Hist. U. S., Vol. IV., p. 351.
Obelia plana Haeckel, vide supra.

There would seem therefore to be more or less uncertainty as to the exact affinities of the species, as will appear in what follows. As to Agassiz *E. plana* there is no description. Hincks' description relates only to the trophosome and is thus far clear and sufficient. But of the medusa he gives no account, and indeed states that it is unknown, or doubtful.

Haeckel's description relates only to the medusa, but is apparently entirely inapplicable to our species. *E. g.*, his species has forty-eight tentacles at birth, and at maturity from 100–120. Diameter of new-born medusa 1 mm., and at maturity 6 mm. Gonads on distal half of radial canals.

The following characters are diagnostic of our species:

In general features quite comparable with other well-known

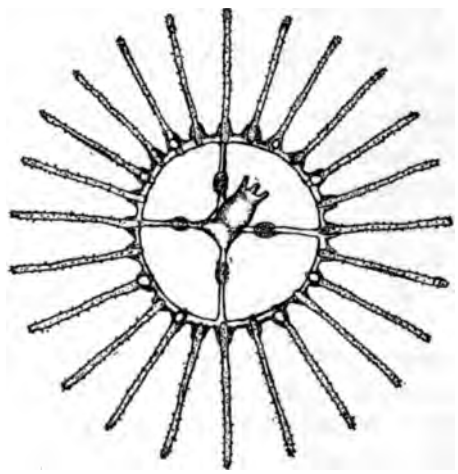


FIG. 12. *Obelia flabellata.*

medusæ of *Obelia*. In common with others the creature has the habit of everting the bell at will, and again recovering its shape Radial canals 4, gonads ovoid in shape and borne about midway between stomach and margin of bell, well-developed at birth. Tentacles 24 in number at birth, number in adult not known. Manubrium rather large, mouth four-lobed. Diameter of medusa at birth .4 to .5 mm. Size in adult not known.

Hence as was suggested above, it seems quite evident that Haeckel's species is not identical with that of Hincks, as shown

by the foregoing comparisons. Fig. 12 gives a general view of the medusa as seen from the subumbrellar surface.

AGLANTHA CONICA. — This species was collected by the writer in August, 1902, and described shortly after (BIOL. BULL., Vol. VI., p. 21). It has not since been taken till the present summer, or spring, when, April 25–30, it appeared in small numbers in the tow at Woods Holl. These specimens agreed in all essentials with those of 1902, except in a slightly larger range of size. In the former specimens the average height of bell was given 5–6 mm. In the specimens of this year, only about a dozen in number, the average was about 8 mm. in height. The marginal tentacles were quite as in the earlier description, and there were no apparent morphological differences between the smallest specimen taken, of 5 mm., and the largest, measuring 12 mm. These facts seem to confirm the previous somewhat provisional designation of the species as new, and warrant the belief that *A. conica* is a well-defined species, distinct from the only other indigenous species, *A. digitalis*. Its occurrence at such widely different seasonal periods as August and April is a point of some biological interest. The gonads were about equally developed in the specimens of each season, and this may also suggest that it has no sharp limits as to seasonal relations.

As a supplementary note it may be added that again specimens have been taken at Crab ledge in late July, 1907, in considerable numbers by towing, as in my original collections, and agreeing in every respect with the original type specimens.

EUTIMA. — Two species of *Eutima* have been recognized by earlier students of Hydromedusæ, namely, *E. mira* McCrady, and *E. limpida* A. Agassiz. In notes by the present writer (*Am. Nat.*, Vol. XXXVI., p. 554), reference was made to *E. limpida* as "Fairly common at Woods Holl, but sexually immature." Upon later and more critical comparison of these medusæ I found it extremely difficult to distinguish more than a single species, and so designated it as *E. mira* McCr. (cf. *Bull. U. S. Bureau of Fisheries*, 1904, pp. 45, 46), though listing *E. limpida*, on the authority of Agassiz, but with the suggestion that it was probably at most but a regional variety of *E. mira*. Still later observations have served to confirm this impression.

LOVENELLA GRANDIS. — This species was first described by Nutting from material dredged in Newport Harbor in 1901. So far as I am aware it has not since been taken. Specimens of the hydroid have since been obtained by the writer at Woods Holl. The colonies were somewhat fragmentary, but still justified Nutting's designation of it as "a beautiful species," and one could wish it were more abundant.

SERTULARIA VERSLUYSI. — For the first time this interesting hydroid has been found at Woods Holl during the present summer. Its habitat on *Sargassum* renders it not unlikely that this is not the first time the species has drifted into these waters, but failed of recognition. Congdon has also taken it on drifting *Sargassum* at Bermuda. A feature of the specimens taken by me, and not mentioned by other observers, is the presence of stolon-like outgrowths from the tips of branches and stems. These are more or less common on my specimens, and it seems somewhat strange that they should not have been observed before.

Rhegmatodes. — This beautiful medusa (*R. tenuis* A. Agassiz), occurring at very irregular periods, and in a very erratic manner, made its appearance during the first week of September, 1907, in considerable numbers and in its characteristic manner. During the last week in August a very few small and immature specimens were taken in the tow, and suddenly large numbers of full-grown medusæ appeared in Buzzards Bay adjacent to Woods Holl, hundreds of which were obtained with dip nets. On the following day not a single specimen could be found. The presence of wind and some rain may have been influential in some measure in the sudden disappearance, but perhaps not entirely. This is the first time the species has been seen in numbers since the summer of 1900.

CAMPANULARIA. — In assorting some specimens in the collections of the Fisheries laboratory a single valve of *Modiolus* was found on which several specimens of a species of *Campanularia* were found which at first inspection seemed rather strange. A closer examination showed them to belong to the species *C. verticillata* (Linn.).

While Nutting lists this species in his "Hydroids of the Woods Hole Region," it is apparently on the authority of Verrill, who records the species as having been taken in the region of Block Island, and in Fisher's Island Sound. The specimens hereunder mentioned were obtained by Vinal N. Edwards off Sankety Light, Nantuckett, from a depth of 25 fathoms. This would seem therefore to be the first definite record of the species from the immediate region, if indeed this may be so designated.

A few points as to structural features seem worthy of note. One interesting fact not hitherto mentioned in its morphology, though well known in the fascicled stems of the Plumularidæ, is the intercommunications of the several stem elements by means of strands of cœnosarc, adjacent tubes showing very clearly such connecting strands. This may be seen in the enlarged drawing, Fig. 17. It is most evident near the apical region, and probably occurs during rapid growth. Another point not mentioned, though probably known, is the variable number of stems in a given fascicle. In young specimens the tubes were as few as five, while in others they were more numerous. Fig. 16 shows the general aspects of a colony.

In size my specimens varied from 20 to 30 mm. in height, the stems ending abruptly at the distal end, as shown in the figures.

No gonangia were present on any of the specimens. This fact with that of the small size of the specimens, would seem to indicate their immaturity.

LAFŒA. — On the same shell from which the previous specimens were taken I also found a species of *Lafœa* of whose exact relations I am in some doubt. There were features which at first inclined me to regard it as *L. gracillima*. Further comparison showed still other features allying it with *L. pocillum*. Unfortunately, the specimens had been allowed to become dried up before they were recognized, hence the uncertainty in their identification.

A single gonosome was found encasing in part a stem of *Tubularia* on which the colony was growing, and in part the stoloniferous portion of the colony. Its features were very much like that figured for *L. dumosa*.

PASYTHEA. — Among hydroids obtained from the gulf-weed were found extensive colonies of what seems clearly to be a species of *Pasythea*, a genus hitherto unrecorded anywhere within our northeastern Atlantic coast region. Indeed only a single species of this genus is recorded from American waters (Nutting, "American Hydroids," Part II., p. 75), namely *P. quadridentata* Lamx., specimens of which he reports from the British West Indies. From a careful comparison of Nutting's figures and description, as well as those of Lamarck, Lendenfeld, and others, my specimens seem to have so many points of difference as to suggest the probability of their being undescribed. Hence it seems important that a rather full description be undertaken, which is given below.

FIG. 13.

Trophosome: Colony consisting usually of a simple unbranched stem, varying in height from 4 to 9 mm., or averaging about 6 or 7 mm., and rising from a creeping hydrorhiza which forms an intricate network over the stems and leaves of the alga. The stem is divided into a series of internodes by oblique nodal articulations, as shown in Fig. 13. Each of these internodal segments bears from one to five pairs of opposite, and closely appressed, hydrothecæ, the mouths of which are quite divergent as shown in the figures, especially in internodes having several pairs, the terminal pair of which in such cases are much less so, owing no doubt to the absence of pressure from above. The number of internodes varies greatly on different stems, as is shown in the accompanying table. The average, estimated from a series taken more or less at random, seems to be about five, though this is probably somewhat dependent upon age or conditions of growth. Furthermore, the size of internodes is likewise variable, even more than their number. This is largely a matter of the number of hydrothecæ upon a given internode. A glance at the table, and a comparison of the figures, will make this quite obvious without extended verbal description. Usually

the lower, or basal internode contains the fewer pairs of hydrothecæ, one or two, or rarely three pairs, and the more distal nodes a larger number. But this again is subject to great variations. It might appear as if these nodulated stems were so many expressions of rhythms of growth, in which there would naturally be a gradual increase in the size of successive internodes up to the optimum, followed by a corresponding decrease in size as the organism passed this optimum of activity. And many stems show just this feature in a very striking way. On the other hand there are so many exceptionally different examples as to throw serious doubt upon the suggestion.

Margins of hydrothecæ are usually characterized by two or three, rarely four, tooth-like processes, but occasionally specimens are almost smooth, or entire. There seem to be two or three opercular flaps closing the mouths, though this feature is difficult of certain demonstration.

Gonosome, entirely unknown.

TABLE SHOWING NUMBER OF INTERNODES, AND PAIRS OF HYDROTHECÆ ON EACH, FOR TEN COLONIES TAKEN AT RANDOM.

No. of pairs of hydrothecæ on each internode.	I.	II.	III.	IV.	V.	VI.	VII.	Number of Internodes.
	2	3	3					
	1	4	3	4				
	3	4	2	5				
	3	2	3	with terminal stolon				
	2	3	3	4	4	3		
	3	3	3	3	4	4	3	
	2	3	3	3	5			
	2	3	5	4	2	4		
	3	4	3	4	4	3	3	
	2	3	5	4	3	with two terminal stolons		

Among the more distinctive differential characters of the species as compared with *P. quadridentata* are:

1. The apparently larger number of internodes to each stem, seven being not uncommon, and in one case eight were present. In *P. quadridentata* the number as given by Nutting is two, or occasionaly three or four.

2. The larger number of pairs of hydrothecæ to each internode. This feature will be quite apparent from the table. In the other species the number is only two or three.

3. The rather stouter and shorter nodal portion of the stem. Nutting's figures show these much more slender and long.

4. The tendency in certain stems to form divergent branches from the sides of the internodes, as shown in Fig. 15.

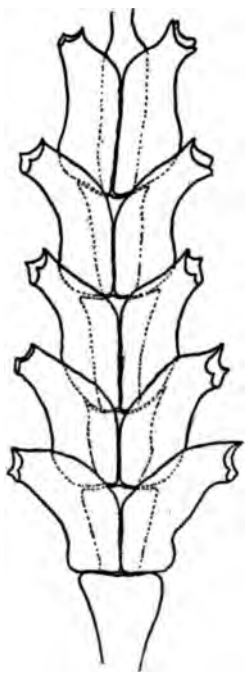


FIG. 14.

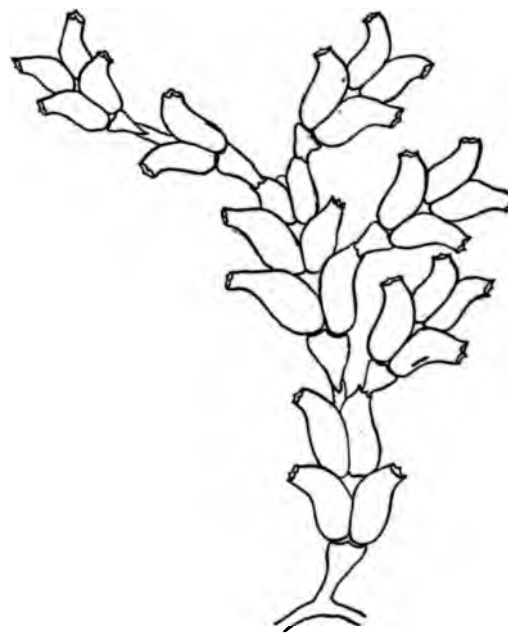


FIG. 15.

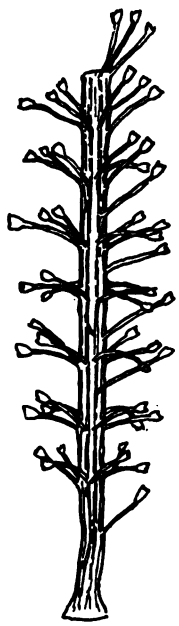
5. The very common and rather distinctive occurrence of terminal and lateral stolons on the stems, from which at irregular intervals secondary stems arise.

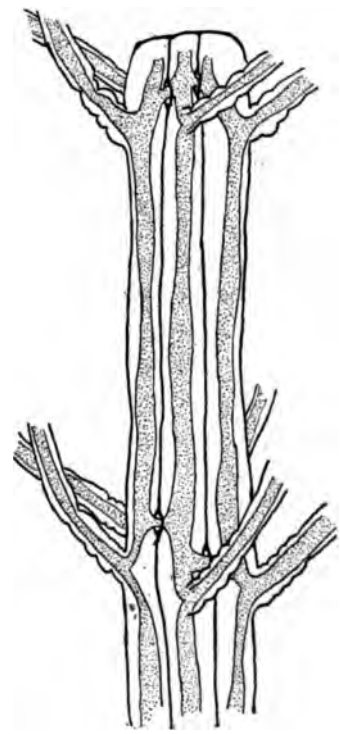
It is much to be regretted that no gonangia were present on any of the specimens, as this would have afforded almost certain proof as to the affinities of the species. It may be found that when specimens are obtained from other localities and in larger numbers, though the numbers in the present case were large, the present species will have only a varietal importance. Still, I am

strongly inclined to believe that it is quite distinct and probably new, and hence will propose for it the name *Pasythea nodosa*, the name being suggestive of a highly distinctive feature of the hydroid, namely the nodose aspect of the stem.

Fig. 14, single internode enlarged. Fig. 15, colony with branches.

Distribution and Habitat.—Known only on floating *Sargassum.*





Campanularia verticillata.

FIG. 16. FIG. 17.

ANEMONIA SARGASSENSIS. New species.

During the summer a very interesting species of actinian was found by the writer in a mass of *Sargassum* brought in by Mr. Edwards from the sound, and in working over some material collected during the preceding summer other specimens were found to have been taken by Mr. Edwards and preserved in

formalin. An examination of the literature at hand failed to give any clue as to their specific, or even generic relations. Subsequent investigation pointed to more or less close affinities of the genus *Anemonia*, of Risso. But there seems little doubt of the fact that the species has not hitherto been described. The following summary of characters will suffice for its general features, and a more detailed account of its anatomical features will shortly be published in another communication.

Column short and irregular in shape, about half as high as broad. Column obscurely fluted, and with pedal disk well developed and adapted to clasping the sea weed, to which it adhered with tenacity. Oral disk characterized by a series of white or cream-colored radiating lines extending from the mouth to the bases of tentacles or even slightly outward on the bases of the larger ones.

Tentacles cylindrical in shape, but slightly contractile, though more or less prehensile at the distal portions; the number varying with size, from 25 to 30 in smaller specimens, to about 50 in the larger, and in length from 5 to 15 mm., tapering to acutish ends, the inner series nearly twice the diameter of disk. The tentacles also are definitely adhesive, sticking to anything with which they come in contact. In several cases bifurcated or forked tentacles were found, adding to the more or less irregularity and asymmetry of these organs.

There are no acontia, nor marginal tubercles. Color a more or less diffused brownish or chestnut, variegated by the whitish markings on tentacles and disk, giving to the creature a close resemblance to the olive brownish color of the *Sargassum*, and rendering its presence difficult of detection. It shares this feature in common with perhaps the large proportion of the fauna of the *Sargassum* forest.

Supplementary Note.

Since the foregoing notes were written and about to be sent to press I have received Hartlaub's admirable paper "Craspedote Medusen" (Part XII. of Nordisches Plankton), in which he discusses certain problems relating to *Gemmaria* and *Zanclea*. With most of Hartlaub's views my own will be seen to be in substantial agreement. Concerning his contention as to the

identity of the genera *Gemmaria* and *Zanclea* of McCrady and Gegenbaur I should have to dissent, at least until such time as more convincing evidence than is at present adduced shall be submitted. Reasons for this will be seen in what has been said in the sections dealing with these subjects. It seems desirable, however, to offer a few additional suggestions bearing upon the matter.

Admitting the doubt expressed by McCrady as to the distinctness of his proposed genus, which was only what might have been expected under the circumstances, the fact must not be overlooked that subsequent students of medusæ have generally accepted without hesitation the validity of McCrady's genus. A. Agassiz who was quite familiar with the medusæ of *Gemmaria* has described a second species under it, namely, *G. cladophora;* and in a comparison of *Gemmaria* and *Zanclea* says: "The form of the bell, of the digestive cavity and of the tentacles are totally different in the two genera" ("Cat. N. Am. Acalephæ," p. 185).

Haeckel also has always recognized the distinctness of the two genera, and has himself described a new species under *Gemmaria*, namely, *G. sagittata.* He says: "Up to the present time the genus *Zanclea* has been represented only by a Mediterranean species, *Z. costata* from Messina. L. Agassiz added to this two other species, *Z. ambigua* and *Z. gemmosa.* Of these however, the first is to be placed under *Pteronema*, the latter under *Gemmaria.*" ("Syst. der Medusen," p. 102.)

In Hartlaub's revised definition of *Zanclea* he has naturally enlarged that of Gegenbaur in order to include under it medusæ hitherto described under *Gemmaria.* But in so doing he fails to submit any grounds of sufficient warrant for a measure thus radical. Let it not be overlooked that thus far the ontogeny of Gegenbaur's medusa is wholly unknown. And furthermore, that even the medusa itself has been rarely seen, or certainly identified by later naturalists.

I do not overlook the circumstance that Browne has described specimens of *Gemmaria implexa* having four tentacles, which he seems to regard as older specimens of those which when first liberated have but two tentacles. This however is merely an *inference*, for he has not traced these four-tentacled specimens to

their hydroid, and until this is done the matter must remain an open question. The same objection must be urged concerning a similar inference on the part of Hartlaub, in which he would identify Gegenbaur's *Z. costata* with that described by the writer at Naples and referred (doubtfully) to *Gemmaria implexa*. Therefore the attempt to identify and unite *Gemmaria* with *Zanclea* must be regarded as unwarranted and inadmissible, and hence premature.

Stanford University
FEB 15 1908

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BIOLOGICAL BULLETIN

THE ANNULUS OF A MEXICAN CRAYFISH.

E. A. ANDREWS.

It is well known that the females of the common American crayfishes of the genus *Cambarus* possess a peculiar structure called in systematic descriptions the "annnlus ventralis." In our common northern crayfishes it has been shown (1) that the annulus contains an essential accessory reproductive organ, the seminal receptacle, without which the species would become extinct. Ortmann has recently divided the genus into six subgenera (2) and also (3 and 4) given reasons for supposing that the seventy or more species of *Cambarus* now living in the United States and adjacent territory are descended from forms once living in the region now called Mexico. As some of the more primitive subgenera are found only in Mexico and as it is not known whether the annulus in any Mexican crayfish contains a reproductive organ or not it seems worth while to describe the real character of the annulus in any Mexican crayfish that can be obtained.

The question as to the evolution and spread of the genus *Cambarus* cannot be completely answered till it is shown that the annulus in all members of the genus does contain the sperm receptacle, and that they thus differ from all other crayfish the world over.

The crayfishes whose annulus is here described were bought in the market of the city of Mexico, in July [1] and prove to be *Cambarus montezumæ* Saus. of the typical form.

This species was first described in 1857 by Saussure (5), but

[1] These specimens were obtained by Horace Andrews, C.E., with the aid of W. W. Blake. They were on sale, cooked, as taken in Lake Zumpango and known by the Astec name "Acociles."

without mention of the annulus; subsequently Martens (6) described a variety and Faxon (7) gave a new account of the typical form, of which he says the annulus "is movable, fixed only at the posterior end, between the sterna of the penultimate and last thoracic somites. The ventral face of the annulus is marked by a longitudinal fossa open at the posterior end" (page 122). Several varieties of the species were later briefly described by the same author (8). Recently Ortmann has examined the specimens of Mexican crayfishes from the Paris Museum (2) and mentioned in a footnote that the peculiar spine posterior to the annulus in his new subgenus *Paracambarus* distinguishes this from all other Cambari except *C. montezumæ*.

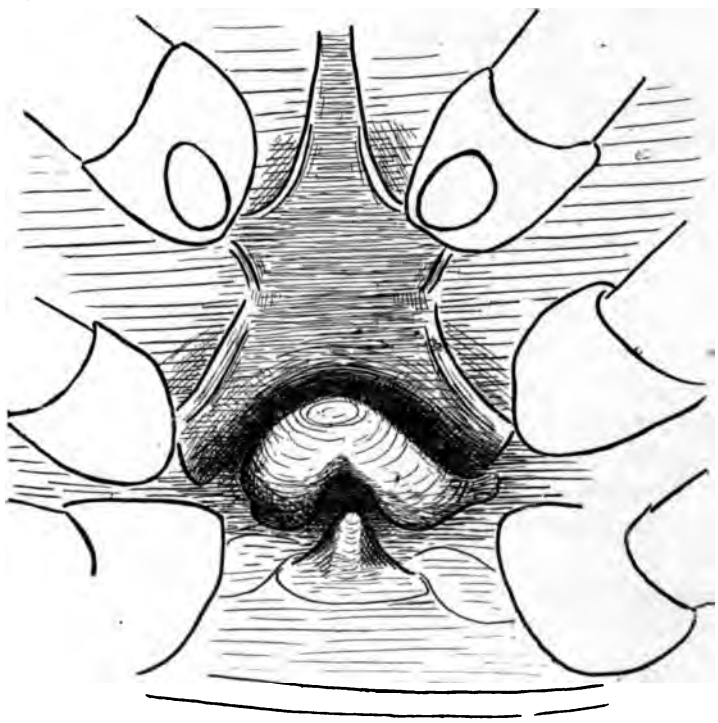

FIG. 1.

The above is all thus far known regarding the annulus in *Cambarus montezumæ*, or in the subgenus *Cambarellus*, in which it is placed by Ortmann.

Of the 179 specimens sent to me 88 were females ranging in length from 16 mm. to 36 mm., many being 25–28 mm. The males ranged from 14 to 36 mm. and many were 25 mm. Saussure had 25 specimens, which measured up to 27 mm., while Faxon's largest was 35 mm. That those 30–36 mm. were sexually mature was evident from the fact that six bore eggs, and two, young larvæ, attached to the abdominal appendages, as in other crayfishes.

The general appearance of the annulus in these small, but mature forms is indicated in Fig. 1, which shows the bases of the third to the fifth thoracic legs and the sternal surface between them. While the annulus has the same position as in all other Cambari, between the sterna of the fourth and fifth legs, it differs from any hitherto figured in not being a low transverse plate of more or less annular form but in forming a high transverse papilla of asymmetrical form.

The sternum between the fifth legs is also more specialized than in higher Cambari in that it projects as a stiff spine, somewhat like a lengthwise ridge, but often more like a conical spine.

This spine fits into a very marked median groove on the posterior side of the annulus in the manner mentioned by Ortmann (2) as characteristic of his new form, *Paracambarus paradoxus*. The eggs issuing from the elliptical openings on the third legs (Fig. 1) doubtless flow back around the base of this rounded, papilla-like annulus, and there receive the fertilizing sperm, for we find in this annulus a functioning sperm receptacle.

The appearance of the annulus when cut off and viewed from the posterior side is shown in Fig. 2. The right and left of the high papilla are unevenly balanced about the long deep median groove and on the observer's left, which is the animal's right, there is a peculiar structure, which proves to be the seminal receptacle. The asymmetry of the annulus is marked, the apex being to one side and the side containing the receptacle being abrupt while the opposite one slopes gradually.

In the crayfish hitherto studied the receptacle is wholly, or in part, on the median line but here we find it entirely to one side. In those crayfish there is, as far as studied, a peculiar dimorphism amongst the females (9), some of them having the entrance of the

receptacle on the right side of the body and some on the left. In this Mexican crayfish it is surprising to find that the dimorphism

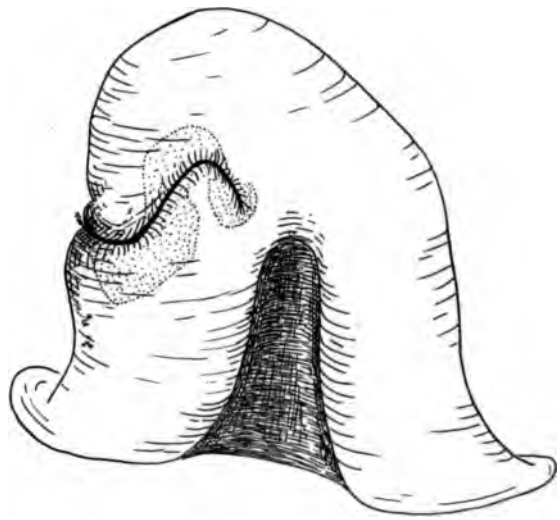

FIG. 2.

is expressed by a transposed position of the entire receptacle. As it happened just forty-four of the females had the receptacle on

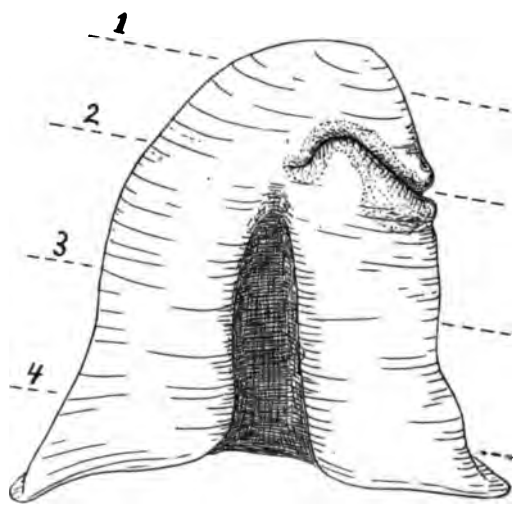

FIG. 3.

the right as in Fig. 2 and the other forty-four on the left. In these left-handed females the posterior side of the annulus appeared as in Fig. 3. Leaving out of account the differences in height and width and the altitude of the receptacle, which are diverse in individuals either dextral or sinistral, the annulus in Fig. 3 is the mirror image of that in Fig. 2 : the abrupt and the slop-

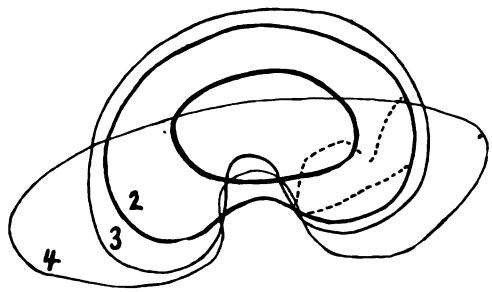

FIG. 4.

ing sides and the position of the apex are reversed and this is obviously associated with the fact that the receptacle is on opposite sides in the two cases.

The real shape of the annulus in either sinistral or dextral forms is seen only after observation from different points of view. The annulus is wide from side to side and compressed from before back, very protuberant in front and less so behind and has a very small base, which accounts for the movability noticed by Faxon. A view of the sinistral annulus (Fig. 3) taken from the side that bears the receptacle is shown in Fig. 5, which emphasizes the very narrow base and the greatly protruding anterior face. The apex is also seen to rapidly taper. The receptacle obviously extends around onto this lateral side above the level of the central groove, which is indicated in broken lines as seen through the side. Upon looking down upon this same sinistral annulus

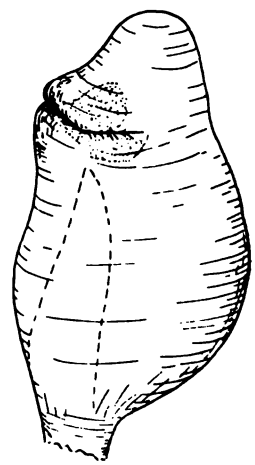

FIG. 5.

from above, successive optical sections presented the outlines seen in Fig. 4: the heavy black line is the outline of the apex above the receptacle; the line No. 2 is the circumference of the annulus around the level (2) of Fig. 3, and in the receptacle is represented by dotted lines; the line 3 shows the maximum thickness from before back, also the greatest depth of the median groove; finally the line 4, Figs. 3 and 4, shows the great width of the annulus and its compression from before back, near its base. An anterior view of this same annulus was like Fig. 3 but with the sides reversed and with the annulus and median groove invisible until the annulus was made transparent, when they were seen through its substance.

By study of annuli cleared whole and also cut into sections the internal structure was seen to agree with what was made out for other annuli (9). Thus a lengthwise section (Fig. 8), shows a thick exoskeleton lined by nucleated epidermis and a central mass of areolar connective tissue full of blood sinuses and vessels with no discovered musculature. The shell on the straighter posterior face seems thicker than on the more protuberant anterior face. The infolding near the upper end was the part of the receptacle of this dextral annulus, cut near the right face.

As a protruding mass of connective tissue covered with thick exoskeleton the annulus in other crayfishes forms a stiff plate more or less movable since the exoskeleton round about it is less thick and more pliable, but in *Cambarus montezumæ* the mobility is apparently enhanced from the fact that the great height of the mass and its narrowed base make it easy to rock it back and forth. The convex front face of the annulus fits against the hollowed out sternal plate of the somite bearing the fourth legs, as s imperfectly indicated in Fig. 1.

As the spine on the middle of the sternum behind the annulus is on that somite of the thorax which can be independently moved by the animal, it may be that the annulus is sometimes caught between the spine that enters its posterior groove and the solid sterna in front of it and subjected to pressure and this may be a means of emptying the receptacle.

The receptacle itself proves to be made precisely along the same lines as in the other crayfish in which it has been described

(9): it is a rather simple infolding of the epidermis, lined by the exoskeleton and is a mere flat pocket opening to the exterior by a narrow chink.

The mouth of the sperm pocket is the sinuous line seen in Figs. 1, 2, 3 and 5, passing as an S-shaped line from the neighborhood of the median groove to the right or the left as the case may be, across the posterior face and then around onto the lateral face of the annulus. It is not extended onto the anterior face. For most of its extent the line is a closed suture, or mere morphological mouth, forming a chink that is apparently closed up, but toward the edge of the posterior face and on the lateral face the chink is more patently an opening into the interior. The lips of this suture are more or less swollen especially on the lateral face and toward the edge of the posterior face and this is much more pronounced in some individuals than in others (Figs. 2 and 3).

The internal pocket into which the suture leads may be seen through the exoskeleton when properly prepared and, as indicated in the above figures by the dotted areas, it extends out rather far on either side of the suture in a peculiar way.

A good idea of the shape of the internal pocket may be got by looking at it from the inside, as in Fig. 6, that is, by removing the anterior walls of the annulus and all the connective tissue and epidermis; the exoskeleton that lines the sperm pocket stands forth as a prominent ridge passing sinuously from side to side. Fig. 6 is such an anterior view of the exoskeleton of the sperm-pocket of a right-handed annulus, similar to Fig. 2. The flat, sinuous ridge there indicated is attached all along the posterior side of the annulus and free anteriorly, toward the observer; the part to the right is the beginning of the pocket on the lateral face of the annulus and stands forward as do the middle and the left parts, forming three roughly parallel parts of an S.

FIG. 6.

The extreme tip to the left, as well as two intermediate regions, are lower and are bent alternately down, up and down. Thus one sees on the right only the top of the ridge, then part of its upper or distal surface, then the top, then part of its lower or proximal surface, then the top again and finally the upper surface at the end. By this mode of bending as a warped surface the edge of the pocket, or ridge, toward the observer is longer than the anterior edge, which is the mouth, so that the same condition is found as in higher crayfish and the sperm pocket is like an elastic coat pocket which should have its mouth bent in an S and its bottom pulled out into an S with longer loops, after the fashion of a mesentery that has a shorter origin and a longer line of attachment. Seen on the outside (Figs. 2 and 3) this greater bending of the internal than of the external

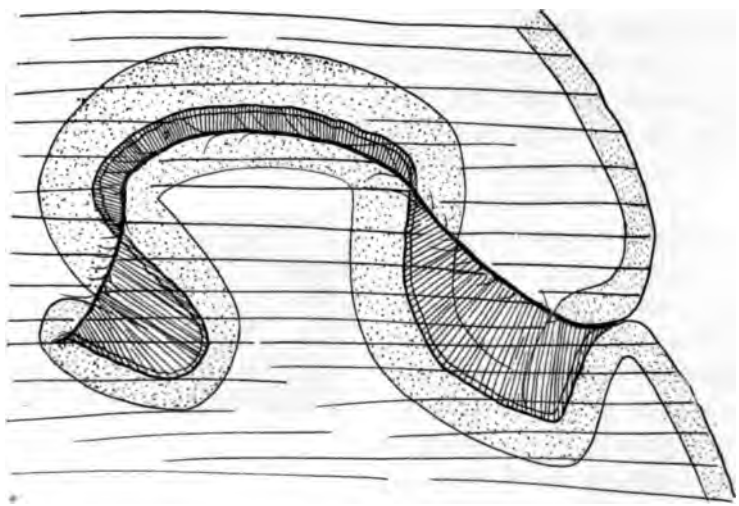

FIG. 7.

limiting edge is evident and variable. In old well-marked annuli the pocket (Fig. 2) drops down from its mouth in a long sweep toward the base of the annulus and then sweeps up above the suture toward the summit to return again toward the median end of the suture. In other cases (Fig. 3) the pocket is less drawn out toward the summit and stands more at right angles to the surface. When more magnified such a receptacle seen from the

outside as a translucent object is represented in Fig. 7. The line of the mouth coming around from the left face makes an imperfect S which is much shorter than the inner edge of the pocket walls, indicated as a continuation of the outside shell. Through the thick exoskeleton runs only a narrow crevice the outer mouth of which is shown as the black line or suture and the bottom of which is indicated by two parallel lines which cross the suture at two points. The sides of the chink, or cavity of the pocket, are diagrammatically represented by ruled lines. The cavity of the pocket thus runs in from the mouth at first obliquely downward and to the observer's left then slightly upward and then downward again and toward the observer's right.

In other cases (Fig. 10) the middle loop toward the apex of the annulus is much more pronounced. The simple nature of

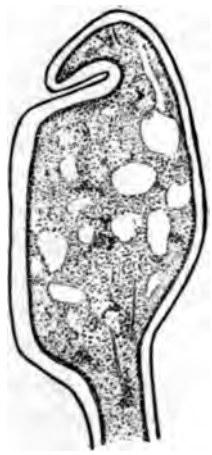

FIG. 8.

FIG. 9.

the pocket is shown in the lengthwise section of the annulus (Fig. 8) which shows the narrow cavity of the pocket of a dextral annulus near its origin toward the right face of the annulus.

Other sections show the same simple pocket, whenever cut at right angles to its twisting course. In Fig. 9 is shown, enlarged, the terminal part of a dextral pocket cut near its median termination where it slopes away from the surface toward the median plain and also down toward the base of the annulus. Here as elsewhere the pocket is seen to be really an invagination of the

epidermis lined with exoskeleton that has the same shape. By careful dissection this epidermis was removed in other cases as a hollow mould in which the exoskeleton had fitted.

That this thin, flat slit in the shell of *Cambarus montezumæ* actually is used as a sperm reservoir is well proven on two specimens that show the sperm as it was issuing out of the mouth of the pocket. One of these (Fig. 10) was a dextral annulus like Fig. 2. In this figure the bottom of the pocket is represented by dotted parallel lines and the exoskeleton by parallel rulings. The outer mouth or suture is shown as a black line. Extending out from this on its middle loop is a large area covered with dots and these were round, flat, clear bodies which with 6 and 2 mm. objective clearly showed the characteristic central bowl of crayfish spermatozoa. These sperms were issuing out of the middle loop of the suture and spreading on either side over the surface of the annulus where they might meet the eggs (Fig. 1). The sperm seemed to be in a single layer over a considerable area, though in a deeper mass where emerging from the suture. They lay flat side by side and their clear outer part around the central bowl suggested that they might be gliding along somewhat like a liquid wetting the annulus. No radiating arms were made out, so that here again the male must have succeeded in transferring the sperm into the annulus without the expansion of the sperms into those stars which were so often produced by the liquids used by investigators as to be regarded as the only shape of the mature sperm. The part played by the male must be the same as in other Cambari for an examination of the first male pleopods revealed some of these same round sperm cells issuing out of the tip of the "canula" or discharging tip of that appendage, which is doubtless inserted into the receptacle.

Whether the above figured sperms were forced out at the time the animals were killed, or just before, is not known, but the appearances are that pressure of some kind must be applied not only to get the sperm into such a stiff rigid pocket (Fig. 9) but also to get it out again. In another specimen the sperm had issued out of the suture all along its median as well as its middle loop and this was probably the case in Fig. 10 before it was examined.

Comparing the annulus of *Cambarus montezumæ* with that of other Cambari we see that though it departs so much in general form yet it agrees in containing a sperm pocket that is both in structure and use fundamentally identical with the sperm pockets of other Cambari. In all cases known the male fills the inner curves of the pocket with sperm and the outermost curve, near the end into which the male pleopod is thrust, with a wax or cement that seals the sperm in.

The sperm pocket here described is strikingly like that previously described for *C. immunis* (9), both in the simplicity and form of its bendings and in the fact that it runs transversely instead of longitudinally as in most crayfish. Again the form of the pocket here described agrees closely with that of *C. clarkii* (9) though the latter is a longitudinal, median pocket.

The sperm pocket of *C. montezumæ* thus resembles the simplest sperm pockets known, those most like an early stage of the more specialized pocket of *C. affinis,* whose ontogeny has been described elsewhere (10).

As far as the sperm pocket is of any value in indicating phylogenetic affinity it points to the conclusion that *C. montezumæ* is not a highly specialized form, but need not be taken to mean that there is any close relation between *C. montezumæ* and either *C. immunis* or *C. clarkii*, which other characters indicate are remote. And the value of similarity in form of the sperm pockets in these three is nullified by their different positions on the annulus. The ontogeny of the sperm pocket in *C. affinis* shows that it starts as a median groove which secondarily has added to it the part right or left of the median line and if this is the general rule for other Cambari such wide departure from the median position as that in *C. immunis* and *C. montezumæ* may well mean remote connection with such a median form as that in *C. clarkii* and if the ontogeny of the annulus in *C. montezumæ* were known the close resemblance of its sperm pocket to that of *C. immunis* might prove to be a superficial one.

As yet of the six subgenera into which *Cambarus* has been divided by Ortmann the sperm pocket has been made known in four: in *C. affinis*, *C. virilis*, *C. immunis*, representing the subgenus *Faxonius;* in *C. bartoni*, representing the subgenus *Bar-*

THE ADHESIVE ORGANS OF AMIA.

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Most of the ganoid fishes have adhesive organs or sucking disks in the early stages of their development.

These organs have received little attention even from morphologists and systematists. Their development has not been worked out with any completeness in any species, but enough has been learned to raise numerous questions. The earliest stages in their development have never been described; even the germ layer from which they take their origin is unsettled. Their relations to other systems, digestive system, nervous system, etc., are in dispute. The various stages in their development, their structure during these stages and the functions they perform, as well as questions of their ultimate fate and their phylogeny and meaning seem to us to merit further study.

In a previous paper ('06) on the "Development of Amia" we have touched briefly on these organs. The present paper is a continuation of the same subject and is based on the same material.

DESCRIPTION OF STAGES.

The anlagen of the adhesive organs appear in the embryo about seventy hours after fertilization, or more precisely, when the embryo extends over about 160 degrees of the circumference of the egg. A median sagittal section of the anterior portion of an embryo of this stage is shown in Fig. 1. The ectoderm consists of a single layer of cells externally (*s.ec.*) and a large mass of deeper lying cells; the deeper layer is some 15 to 20 cells in thickness. In this mass of cells which forms the anlage of the future brain there is a slight cavity (*br.c.*) which is the first cavity to appear in the brain. In front of the brain is a second mass of thickened ectoderm (*p.cb.*) which has been called the precerebral mass. The gut-cavity (*g.c.*) is now widely extended laterally. In its anterior portion its dorsal and ventral walls are

the lateral evaginations of the cœlom which later unites with a corresponding structure on the opposite side to form the heart.

Fig. 5 shows an obliquely transverse section through an embryo somewhat older. The anterior end of the forebrain (*f.b.*) appears as a solid mass of elongated cells. In connection with its ventral wall, the optic stalk passes obliquely outward and terminates in the optic vesicle (*o.v.*). On the other side the section passes through the anterior portion of the adhesive organ (*a.o.*) which here shows its connection with the anterior end of the foregut (*g.*). The foregut is almost closed off ventrally through the approximation of the cœlomic cavities. Between the end of the brain and the optic vesicles there is a slight invagination (*n.*) of the deep ectoderm to form the beginning of the nasal pit. The character of the epithelium of the adhesive organ is now more plainly shown owing to the absorption of yolk granules. It will be noted that the adhesive organ is now in contact with the superficial ectoderm (*s.ec.*) which extends over it as a double layer.

Another section through an embryo a few hours older taken in a sagittal plane is shown in Fig. 6. The section passes through the embryo in such a direction that one of the adhesive organs is cut lengthwise. The organ at this time is still in open connection with the gut. Its antero-dorsal surface follows closely the external contour of the embryo. The peripheral end of the organ thus extends both posteriorly and mesially following the course of least resistance. In many of the sections of this stage, or slightly later, a constriction is formed around the middle of each of the large organs which soon gives rise to a pair on each side of the snout.

The minute structure of the organ is very similar to that shown in Fig. 5. It is composed of very high columnar epithelium that presents a pseudo-stratified appearance. These columnar cells are heavily laden with yolk granules which are very large and dense at the bases of the cells but gradually diminish in density and size toward the inner ends of the cells until these ends become free from yolk. These yolk-free ends show that cellular metabolism is here most active. The indications are, from the condition of the reticulum and the stainable fine granules, that they are secreting cells. Moreover the presence of small droplets on the surface of the cells adds confirmation to this view.

will show. The ectoderm (*s. ec.*) has undergone considerable thickening and differentiation. It no longer extends over any portion of the organ and as a result a far greater number of cells now reach the surface. The character of the cells is somewhat different as a result of the general change in the form of the organ. They are now typical, pseudo-stratified columnar cells while in preceding stages the outer ends were compressed. The cells no longer possess yolk granules while the prozymogen granules show more plainly in the outer ends. That some sort of a mucous secretion is furnished by these cells is indicated, not only by the cellular structure but also by the fact that when the larvæ are detached from the various objects, to which they now adhere, it is often observed that fragments of aquatic plants cling to the organs.

The larva of 13–14 mm. begins to move freely from place to place. It rarely attempts to attach itself as in the preceding stages. Corresponding with this change in the behavior of the young fish there is a marked change in the position of the adhesive organs. When viewed from the surface, some of the disks forming the horseshoe are still distinctly visible while others are barely discernible. All, however, show a great reduction in number when compared with the larva of 8–9 mm. A section of one of these organs is shown in Fig. 10. It will be noted that the entire organ has now sunk below the level of the epidermis and is partly surrounded by dermal pigment. The organ is still in communication with the exterior through a funnel-shaped opening in the epidermis.

In the larva of 18–20 mm. the organs have sunk still deeper below the level of the epidermis. Some of them still communicate with the exterior, while others are completely covered by the epidermis. Those that communicate with the exterior show a long narrow epithelial tube with numerous branches or diverticula at its inner end. The walls of these tubes are several layers of cells in thickness as shown in Fig. 11. Toward the inner end of the tube the epithelium becomes thinner until there remains but a single layer of cells covering the smaller branches. This layer becomes broken up in the smallest branches and these epithelial cells become swollen, the nuclei show chromato-

mass of the cells being filled with a protoplasmic reticulum. They may probably be regarded as modified mucous cells." In regard to its function they say: "It does not appear probable that the disc has a true sucking action. It is unprovided with muscular elements, and there appears to be no mechanism by which it could act as a sucking organ. We must suppose, therefore, that its adhesive power depends upon the capacity of the cells composing its papillae to pour out a sticky secretion."

Von Kupfer ('93) has described the development of the head in *Acipenser* basing his studies on median sagittal sections. He derives the adhesive organ from the inner layer of the ectoderm and states that it arises in close connection with the hypophysis. It is at first single but becomes paired by a groove on the median line. Each of these disks divides again, forming four papillæ. These four papillæ develop into the four barbels of the adult fish.

In regard to his derivation of the adhesive organs from the ectoderm we may say: (1) Kupfer's work is based on median sagittal sections and such sections are not those best adapted to trace out the development of a paired organ like the adhesive organs. (2) He has not described the earliest stages. His youngest stage is an embryo forty-five hours after fertilization. (3) Even by Kupfer's showing these organs are connected with the digestive system, though it may be only through the hypophysis.

Ehrenbaum ('94), as stated by Ziegler ('02, p. 157), gives a different origin for the adhesive organs in *Acipenser*. We translate from Ziegler: "On the under side of the head in front of the mouth one finds on each side two thickenings (Wülste) which become the barbels. At this place we noticed in a somewhat earlier stage a depression marked by a peculiar pigmentation; this corresponds to the sucking disk of *Lepidosteus* and *Amia*." How this is to be reconciled with von Kupfer's account of a common anlage for adhesive organs and hypophysis is not apparent to us. Unfortunately Ehrenbaum's paper is not accessible and we are not in position to give any further account of his work.

On the whole then, while we have the high authority of von Kupfer in favor of the view that in *Acipenser* the adhesive organs take their origin from the ectoderm, in our opinion the subject deserves further investigation as the proof is far from being conclusive.

to the germ layer from which these organs are formed. But we cannot agree with her statement that the organ consists at first of a single diverticulum which subsequently divides into two. We find the anlagen of the adhesive organs in paired thickenings of the dorso-lateral portion of the anterior extremity of the foregut (Fig. 3). As soon as these become recognizable as diverticula there are plainly two of them.

As to their method of disappearance we are unable to find anything which indicates an infiltration of leucocytes or phagocytosis. On the other hand everything suggests an autogenous degeneration.

It has been shown beyond question that in *Amia* the adhesive organs are developed from the entoderm. But in the other ganoids, *Acipenser* and *Lepidosteus* — while the proof is not conclusive — the balance of authority favors the view that they are formed from the ectoderm. *A priori* it does not seem probable that homologous organs would be formed from different germ layers. But what proof have we that these organs are homologous? Sucking disks are formed in many species of animals in widely different groups. Generally they are temporary larval organs, and it can be easily shown that they are not in all cases homologous organs. In those amphibia which have sucking disks these organs are undoubtedly ectodermal in origin. In the teleost *Echeneis* (*Remora*) the great sucking disk — an adult organ in this case — is developed from the anterior end of the dorsal fin. Ascidians also develop sucking disks; they too are ectodermal.

As to the function of these organs all authorities seem to be agreed. Their function is to attach the young fish to some solid object. As a rule the organ is well developed when the fish is hatched and the little fish attaches itself immediately. This is true at least of *Lepidosteus* and *Amia*. In *Amia* the organ serves to keep the young fish attached for about a week; in *Lepidosteus* about two weeks.

Whether the organ has now or ever has had any other function has not been shown. It has been suggested that they may serve to convey some sort of nutriment to the digestive tract but we have found no proof of this. It has also been suggested that

they are modified sense buds but this is certainly not the case in *Amia*. But the fact that in *Acipenser* the adhesive organs become tactile organs, put with von Kupfer's statement that the adhesive organs and the hypophysis have a common anlage may suggest some remote connection with the nervous system.

As to the phylogeny or meaning of these organs facts are too few to form a basis for any very extensive or certain conclusions. In the various species of ganoids they may have a common function; whether they have a common origin is doubtful. They do not have a common fate. In *Amia* they disappear entirely. In *Lepidosteus* they leave only a useless rudiment. In *Acipenser* they become the barbels. This last fact may furnish a clue to the origin of the barbels in the teleosts as suggested long ago by Balfour. The adhesive organs of ganoids — if we may base our conclusions on their development in *Amia* — are not homologous with the sucking disks of the amphibia or the teleosts. So far as we can see now these organs were developed independently in different groups of animals. But living forms of ganoids are so few and so aberrant that any conclusions based on the study of these forms alone will probably be only tentative.

SUMMARY.

The development of the adhesive organs in *Amia* begins about 70 hours after fertilization when the embryo extends over about 160 degrees of the circumference of the egg.

They appear as paired thickenings of the dorso-lateral portion of the anterior end of the foregut. They are therefore entodermal in origin.

They grow out as paired diverticula of the foregut, break through the epidermis, become greatly subdivided and form a horseshoe-shaped structure at the end of the snout.

Histologically they consist of high columnar epithelial cells. These cells are at first heavily laden with yolk granules especially at their base. They contain a reticulum coarser at the base. Metabolic activity is at first greater at the basal end of the cells but later it is greater at the distal end.

The function of these cells is undoubtedly to secrete a mucous substance by means of which the young fishes attach themselves

to plants or other fixed bodies. They function as secreting organs till they sink below the epidermis.

The organs reach the highest stage of their development just after hatching. They are functional for about a week and disappear in two or three weeks. The mode of disappearance is probably by cytolysis.

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ABBREVIATIONS.

a.o.	adhesive organs.	*g.en.*	gut entoderm,
br.c.	brain cavity.	*h.b.*	hind brain.
c.	cœlom.	*ht.*	heart.
ch.	notochord.	*mes.*	mesoderm.
d.ec.	deep ectoderm.	*n.*	nasal pit.
en.	entoderm.	*o.v.*	optic vesicle.
f.b.	fore brain.	*p.*	pigment.
g.	gut.	*p.cb.*	pre-cerebral mass.
g.c.	gut cavity.	*s.ec.*	superficial ectoderm.
g.d.	gut diverticulum.	*y.m.*	yolk mass.

EXPLANATION OF PLATE VI.

Figures 1–6 are magnified about 50 diameters; figures 7–11 about 450 diameters.

FIG. 1. Median sagittal section of the anterior portion of an embryo about seventy-two hours after fertilization, showing the anlage of the adhesive organs.

FIG. 2. Oblique sagittal section of anterior portion of embryo about ninety-five hours after fertilization.

FIG. 3. Transverse section through an embryo of same age, showing paired diverticula of the foregut which become the adhesive organs.

FIG. 4. Sagittal section of an embryo one hundred and ten hours after fertilization showing median portion of the adhesive organs and the heart.

FIG. 5. Obliquely transverse section of an embryo one hundred and twenty-five hours after fertilization passing through the anterior margin of the adhesive organ on one side and the optic stalk and vesicle on the other.

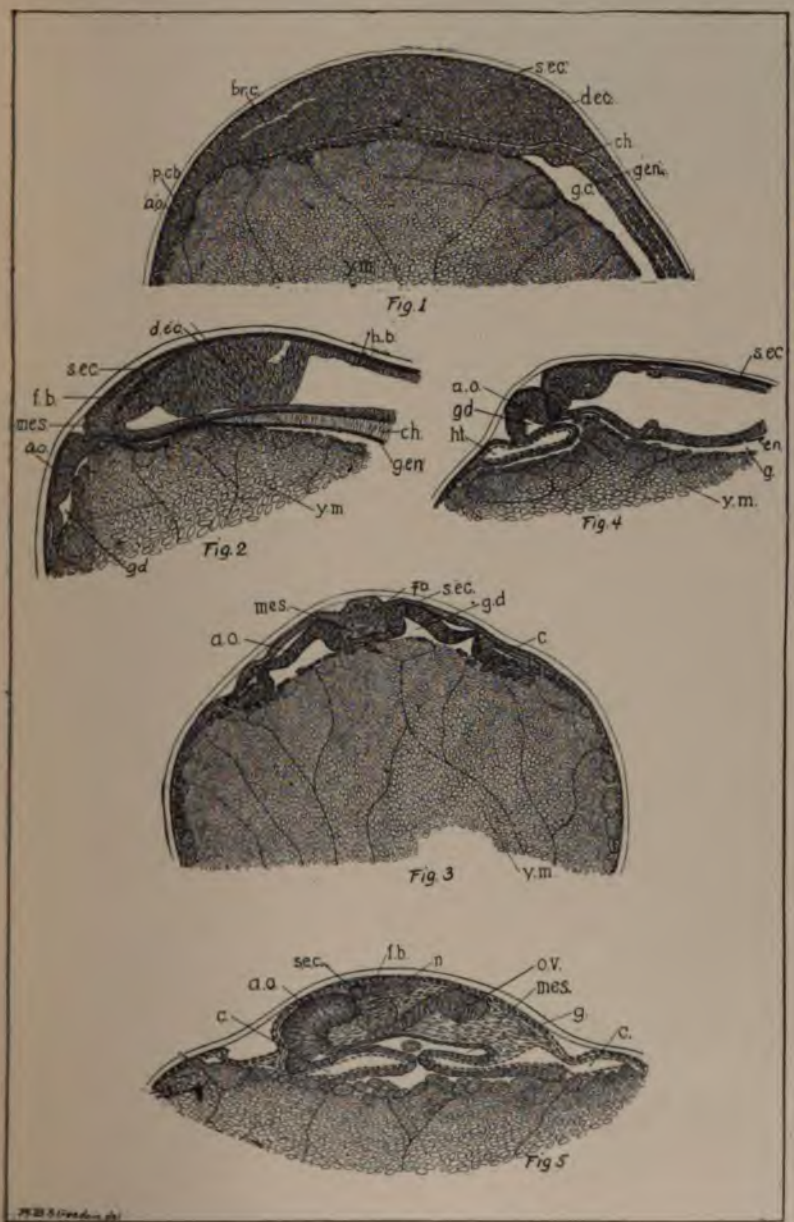

Fig. 1

Fig. 2

Fig. 4

Fig. 3

Fig. 5

EXPLANATION OF PLATE VII.

FIG. 6. Sagittal section through an embryo a few hours older than that shown in Fig. 5, showing one of the adhesive organs cut lengthwise.

FIG. 7. Section through a single disk of the adhesive organ of a larva in which both head and tail are free from yolk.

FIG. 8. Section through a single disk of a larva about 8 mm. in length.

FIG. 9. Section through a single disk of a larva about 9 mm. in length.

FIG. 10. Section through a single disk of a larva about 14 mm. in length.

FIG. 11. Section through a single disk af a larva about 20 mm. in length.

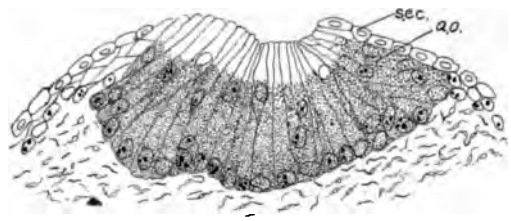

Fig. 8

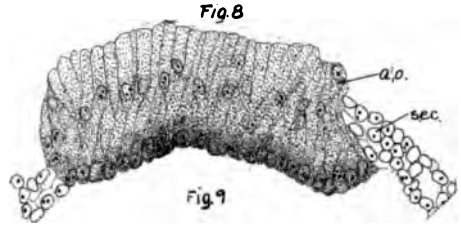

Fig. 9

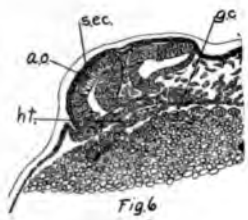

Fig. 6

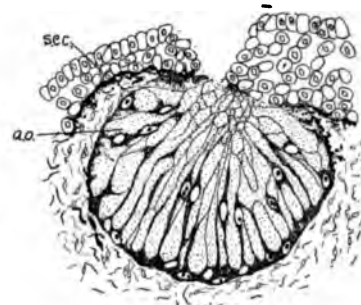

Fig. 10

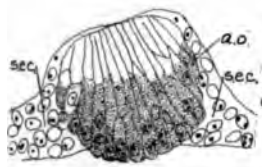

Fig. 7

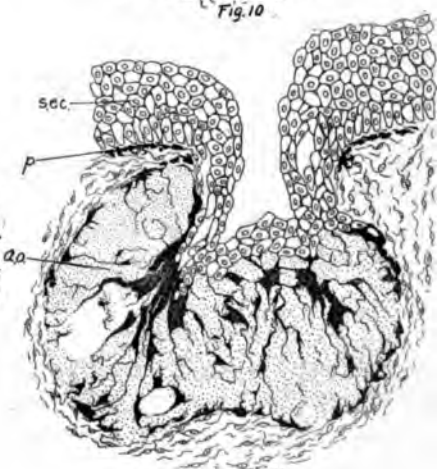

Fig. 11

SOME FURTHER RECORDS CONCERNING THE PHYSIOLOGY OF REGENERATION IN TUBULARIA.

T. H. MORGAN.

The following experiments are in the main continuations of previous ones carried out to test further certain results whose interpretation was in doubt owing to insufficient data. The main problem concerned the influence of regeneration at the oral end on the rate of regeneration at the basal end. Experience shows that caution must be used in interpreting the experiments that bear on this point, because, in the first place, the rate of regeneration is greatly influenced by slight changes in temperature, and therefore accurate results along these lines should be carried out in the future in water kept at a uniform temperature. The difference of temperature of day and night is a serious obstacle when dishes are kept at room temperature. In the second place the aeration of the water is also an important factor and should be controlled. The extent of surface exposure of the water, the number of pieces in a dish, *and the number of other organisms attached to the stems*, also influence the time of regeneration, in the latter cases by utilizing the oxygen or by fouling the water. In the third place there can be little doubt that an internal "condition" of the colonies plays a rôle in the result. Control pieces from the same colony should always be present, but even this precaution is not entirely sufficient, since different stems of the same colony may differ in their internal conditions.

Despite these difficulties some general conclusions may be drawn, although certain of the experiments will be more profitable when the external conditions are more fully controlled. By keeping the dishes surrounded by running water I have met some of these difficulties in many of the experiments where rate of regeneration is involved.

THE SIMULTANEOUS DEVELOPMENT OF ORAL AND BASAL POLYPS.

As previous work has shown, the "rule" for pieces of the stem of *Tubularia* is for the oral primordium of the polyp to develop first, and then, after several days for the basal polyp to develop unless stolons have already appeared; yet not infrequently the oral and basal ends develop simultaneously. In a few instances the basal hydranths may appear before the oral. It was first shown by Driesch and afterwards confirmed by myself that the time of appearance of the oral hydranth depends on its distance from the oral end. The same relation is observed for basal polyps also. It is therefore of some interest to compare the time of regeneration of oral and basal polyps in those cases where both polyps appear simultaneously to see if any acceleration or retardation can be observed.

In one case after 50½ hours 23 of 75 pieces showed the beginning of oral primordia. There were amongst these, three "double" pieces, *i. e.*, pieces with both oral and basal primordia. In two of these the oral end was more developed; in one the basal end was ahead. After another 24 hours there were 43 oral primordia, of which 21 were on "double" pieces. There were also 3 pieces with only basal primordia. Thus at first there was an excess of oral development, but the number of basal primordia increased at the second observation. A large number of pieces had not produced as yet the oral primordia, and three of these pieces had basal primordia only. The results show that both ends may develop simultaneously, and in such cases the rate of development may not be behind that when only one end develops. It is evident therefore that, if the stimulus to develop is present, two polyps may develop at the same rate as does a single one.

In another case, after 45 hours, 56 of 92 pieces had oral primordia only. Twenty-four hours later there were 28 double pieces. In another case after 45 hours, 27 of 55 pieces had oral primordia only. Twenty-four hours later there were 27 oral primordia, and in addition 18 double pieces.

In another case, after 48 hours, 10 of 29 pieces were double; of these 6 were equally developed at each end, 3 had the oral end ahead and 1 the basal. In another case at another time, after

In a third case, after 24 hours, 6 of the distal pieces had oral primordia, and none of the basal pieces had developed. Twenty-four hours later the former had 32 oral primordia and one basal. Some of the basal pieces had developed. Twenty-four hours later still the former had 33 oral, 3 double and one basal primordium ; the latter 8 oral.

In a fourth case, after 48 hours, the distal pieces had 16 oral and two double premordia ; the basal pieces showed 11 oral, 4 double and 6 nothing.

In a fifth case, after 48 hours, the distal pieces had 28 oral and 13 double primordia ; the basal pieces 12 oral and one double primordium.

The results seem to show no acceleration owing to oral development, and on the other hand no necessary retardation if both primordia start at the same time. Local factors must therefore determine the results. Most of the distal pieces served also as controls in cases in which the oral ends of other pieces had been tied. In all such cases there was a very great excess of basal primordia in the orally-tied pieces, showing that there is a distinct effect produced in this way. It would seem therefore that despite the fact that both oral and basal polyps may develop simultaneously, the oral development, if it gets the start, inhibits to some extent the *beginning* of basal development. The nature of this influence is still obscure.

Are the Changes in Isolated Pieces Local or General?

Pieces were tied near the oral end and then after a number of hours as much of the basal end as would contain the primordium of the polyp was cut off. In the first experiment the basal cut was made ten hours after tying. Twenty-three hours after the beginning 3 of 9 pieces showed basal primordia. In the control (tied but not cut) 3 of 9 pieces also showed basal primordia. The removal of the basal piece had not, within a total of twenty-three hours, delayed the basal development. In other words the cut end did in thirteen hours what it took the control twenty-three hours to do. This means that the rate of regeneration was accelerated by changes taking place in the pieces at least some little distance from the cut end. No doubt the development would have

order to see if the amount of materials set free by the breaking down of the ridges near the oral end (preparatory to the development there of a polyp) has an influence on the rate of basal development. As a control some pieces were also tied at once at the same level. One such experiment is shown in the next table.

AFTER 48 HOURS.

Control.	Tied after 6 hours.	Tied after 20 hours.
11 Basal	8 Basal	3 Basal
2 Nothing		

Another experiment gave similar results. In a third experiment pieces, tied near the oral end after 9 hours, produced basal primordia before those tied at once.

In this third experiment there was perhaps some indication that when pieces were left open for a time at both ends and were then tied at the oral end the basal polyps developed sooner than when the oral ends were tied at once. This result I had obtained before. The three following experiments do not give the same result.

Of 12 pieces tied at once at the oral end, 2 had basal primordia after 48 hours. Of 11 pieces tied after 26½ hours 3 had basal primordia. After another 24 hours the former had 5 primordia and 6 polyps, the latter 10 basal primordia (and one nothing). These were less advanced than those tied at once.

In another experiment 16 pieces were tied at once near the oral end and produced after 48 hours 14 basal primordia. Of 8 pieces tied after 26 hours 6 had basal primordia. The evidence seems to show that no retardation occurs (or very little) if the oral ends are tied after 9–24 hours as compared with pieces tied at once, and there may be an actual, *i. e.*, an absolute acceleration.

The results of another experiment are also still doubtful after repetition of it. Pieces were left open for several hours and a ligature was then tied near the oral end in some of the pieces, and in others near the basal end. The time of development of the basal primordia was noted in each case. Previous experiments had given some indication that the longer basal pieces produced primordia before the shorter ones. It seemed not improbable, if this were really true, that the amount of material set free

from the oral end accelerated basal development, for more of it would be shut off in longer pieces, although not proportionally more. The new experiments showed that in some cases the pieces ligated nearer the basal end developed as soon as those ligated more orally. Moreover the ligature itself if too near the basal end may interfere with development there. It appears then that it is still unsafe to draw any conclusions from these experiments.

Stolon Formation.

Stolons develop after several days in a small percentage of cases from the basal ends of pieces lying on their side in the dish. More often the basal ends produce heteromorphic polyps. The absence of stolons from the oral ends was first noted by Loeb. The potentiality to produce stolons must of course be present throughout the piece, since they may form from basal cut ends at any level. I attempted in the following way to produce them at the oral ends by first tying pieces so that basal polyps or primordia appeared, and then by cutting off the piece behind the ligature. The presence of the basal polyp might be imagined to make the conditions favorable for the development of oral stolons. This would certainly be the expectation if the polarity of the whole piece is reversed as Loeb supposed. In no case were oral stolons produced in this way, even when the pieces with basal polyps were cut off near to the polyps.

In one case the pieces were cut off below the ligature 10 hours after tying. After 48 hours from the beginning there were 3 oral primordia and no basal. Other pieces had been cut off after 24 hours (when the ligatured controls showed 10 of 27 pieces with basal primordia). All 14 pieces showed oral primordia, and 8 of these had primordia also at the basal end more developed than those at the oral end. The presence of basal primordia had not delayed the development of oral primordia. In fact the latter appear to have been accelerated — not however necessarily owing to the basal development but to other changes in the piece.

It is probable that external factors combined with the internal factor called polarity has something to do with stolon-formation. But even when the oral ends of pieces with basal polyps were

covered with sand or were thrust into little heaps of sand no oral stolons developed.

Influence of Diluted Sea Water.

Some observations of C. D. Snyder on *Tubularia crocea* of the Pacific Coast seemed to him to indicate that in dilute sea water more basal polyps are produced than in normal sea water. In several experiments that I made with *T. crocea* of Woods Hole in which the sea water was diluted no more basal polyps developed than in the control in sea water. The occurrence of basal polyps is so variable that only a thoroughly controlled set of experiments would suffice to prove that dilution affects basal polyp formation.

Direction of Currents.

In *Tubularia* a current passes up one side of the hollow stem and down the opposite side. When more than one dissepiment is present there may be two currents in one direction and one or two in the reverse direction. Those who maintain (Goebel and Loeb, for example) that currents may determine polarity might hope to find in the direction of these currents in *Tubularia* a solution of the problem of polarity. In fact Loeb has suggested such an hypothesis. Since however there is a current in each direction it is difficult to understand how this idea could be supposed to account for the difference in the behavior of the two ends, unless indeed there is a dorsal and a ventral side to *Tubularia* (which seems to be radially symmetrical) so that the dorsal side of the oral polyp is on the opposite side from that of the basal polyp. This supposition is not however in harmony with the heteromorphosis in other forms, such as the earthworm or planarians. In these the ventral and dorsal surfaces are the same in the old and in the heteromorphic structures. The only alternative would be to expect, if the currents in Tubularia are a factor in the direction of regeneration, to find that the current near the basal, or heteromorphic polyp is reversed in direction as compared with that going to the oral polyp.

I have examined the direction of the current in a number of long double pieces, and have found that the direction is continuous along each half of the piece and not different at the two ends.

that we call polarity. These material differences with their correlated physiological differences lead to differences in the behavior of the two ends, for, while the material exposed at any cross-cut is the same at both ends, it behaves differently on account of the relation of the end to neighboring regions. This relation may be thought of as one of direction and this is polarity.

It has seemed to me possible that the relation of the parts to each other might be expressed as the relative condition of tension — in a physiological sense — that exists between the different parts. Further analysis has led me to think that behind this relation there is a more subtle one and that irritability is the physiological factor that regulates the behavior of the cells in development and in regeneration. Even the differentiation of the different regions must be supposed to be due to their relation to neighboring regions. In fact, one of the first and most obvious changes that takes place in cells when removed from contact with their fellows is a loss of differentiation, followed by a re-differentiation in relation to a new terminal condition. Polarity therefore in the last analysis stands for a graded relation resting on a condition of contractility (tension) that exists between different levels.

The Cause of the Delay in Basal Polyp-formation when the Oral Polyp Develops First.

In my last two papers on Tubularia I have laid perhaps undue emphasis on the question as to how the basal development of a piece is accelerated when the oral end is tied and conversely as to how the basal end is retarded when the oral end develops. The problem interested me because of its apparent wider bearings; for it seemed possible in this case to test by suitable experiments whether the result could be explained on purely chemical grounds or whether a different principle was involved. The attempt to find a sufficient chemical stimulus does not appear to me to have been successful, for the confessedly incomplete data on which my conclusion provisionally rested has not withstood a second attack. It seems to me now not improbable — and more can not at present be said — that the retardation of the basal development is directly owing to the formative changes taking place at the oral end.

closure, disclose several points of interest. The membrane, that forms and advances ring-like toward the center, is relatively thick and is composed of a large number of cells. The slight withdrawal of the cœnosarc from the cut end is insufficient to account for the presence of so many cells, and the only interpretation that remains is that the cells of both ecto- and endoderm must be drawn towards the cut surface for some little distance from the ends, although the closure of quite short pieces shows that the result may also be attained by a shorter length being utilized. This involves a greater decrease in thickness of the neighboring cœnosarc wall.

What factors are involved in the closure? We may suppose that the stimulus of the sea water, or surface tension, or the loss of contact relations of the material, with its concomitant change of tension relations, is the initiatory stimulus. The last seems to me the main factor for reasons given in previous papers. The tension equilibrium lost, contraction follows. The cut edge contracts as a whole, and as a result of the intimate fusion of the outer layer of the ectoderm with the perisarc the diaphragm-like membrane develops; for if separation from the perisarc is first accomplished a different kind of closure takes place. As the process continues the cells are drawn out bodily over the transversely closing membrane. Despite the rather intimate union of the cells with each other they not only change shape but shift their relations to each other, and while at first a considerable number of cells reach the edge of the closing membrane, their number becomes fewer as the opening gets smaller.

Our analysis leads to the conclusion that the closure is a contractile process of the living substance.

When closure is completed the relation of the parts to each other is still an unstable one for the end of a piece. This leads to further changes in conformity with the irritable nature of the materials and a new polyp develops in consequence. If our analysis is correct we are led to the view that the essential factor in the closure of the stem is the irritability of the protoplasm which leads it to undergo movements (through its power of contractility) of a peculiar kind. This same process, that we find reduced to its simplest term in the closure of the stem, is the mainspring

also of all or most of the formative changes that follow. Irritability therefore is the essential factor in formative action and leads first to change in position of the cells and ultimately to their differentiation. Yet the process of closing gives every appearance of taking place independently of the cells as units; for the movements *appear* to take place in the material as a whole and not to be the sum total of a vast array of cell-adjustments. In other words the cells adapt themselves as best they can to the mass action that is going on and do not take the initiative in the process. Nevertheless it is unsafe to argue from such gross effects that cells have no independent functions. Their power to divide at different times, and the cellular limits of differentiation, show their independence in certain respects. If, however, as seems probable, the cells are united by a meshwork of protoplasm the irritability of the living material may act without heed to cell boundaries.

The Nature of Formative Action.

After the closure of the end of the stem of *Tubularia* the condition is still one of unstable equilibrium; by which I mean that the stimuli received at the closed end cause further changes in the relation of the end to the rest of the piece. The stimuli may be entirely or largely internal, resulting from an unstable termination. In *Tubularia* both external and internal factors may act. This relation leads to the formation of a new polyp in which the relation of the materials to the outside world and to the rest of the piece is a stable one. If my analysis of the factors involved, first in the closure of the stem, and later in its further elaboration into a polyp, is correct we are led to the view that the essential factor is the irritability of the material that determines the location of the formative changes. As I have given recently (the Seventh International Congress, August, 1906) my reasons for coming to this general conclusion I shall not enter further into the argument here, but desire only to bring the interpretation into connection with the present results.

The modern school of developmental mechanics has sought to explain formative actions as the result of familiar chemical and physical phenomena but has not met with any marked

success in explaining such actions. The vitalistic view goes to the opposite extreme and postulates unknown or unknowable processes. Both seem to have overlooked the possibility of accounting for formative changes by the familiar processes of irritability and contractility, that appear to be fundamental attributes of living materials. These cannot be reduced as yet to any known chemical or physical phenomena, but neither does it follow that they belong to a vitalistic category.

To sum up: the formative principle as seen in development, growth, and regeneration, appears to be an expression of the irritability of the living material. The problem of formative action is therefore intrinsically one of response to external stimuli or to internal relations between the parts through the agency of irritability. Contractility is one of the most usual methods of responding to the condition of stimulation that exists in a given region, while differentiation follows quickly in its train. The formative principle is the outcome of a factor that is not one of the familiar chemical or physical events.[1] This conclusion does not force us into vitalism; for until we know something of the nature of irritability it is premature to insist on referring it to any larger category. As yet we are only at the threshold of a knowledge of formative changes.

[1] To those who refer the varied manifestations of irritability to a psychical principle the problem of development will appear to belong to that category; but to those who see in irritability only a "physiological process," the same problem will seem to be physiological. The distinction may be verbal and conventional.

writer ('07, '08), will find that Jones' plates and description practically prove the existence of another variety of this same defect; in other words one may say that the modified region of the down — the "quill" — is a variety of defect which we have already recognized in other situations, but which here occurs in a very uniform way — at a point near the end of the feathers of the first plumage. The fact is, simply, that the defective region falls so close to the end of the feather that it does not include any of the shaft of the feather to which it belongs.

There can be, I think, no question as to this interpretation. The exact conditions met with in the defects or fault-bars already referred to are to be found in the modified region of the down: (1) In the modification or absence of barbules (Davies '89, Jones); (2) in the occasional fusion of the barbs (Klee '86, Davies, Jones); (3) in the diminished differentiation (Davies, Jones) and growth (Davies); (4) in a defective development between a more distal and a more proximal part of the same feather (Jones).

Now if the "quill region" of the down is the morphological equivalent of the fault-bars (which are often produced "normally" and which have been produced at will experimentally, at all levels in the definitive feather), we should expect to find that the two have the same or a similar cause. What is the evidence for such a common cause? What causes the production of "down"?

The Cause of the Modifications in the Down.

It has been established beyond question that the several types of fault-bars are produced by insufficient nutrition; even the variations produced in the available food-supply by the daily fluctuations in blood-pressure was shown to be able to leave its mark on feather structure (Riddle '07, '08). What now is the evidence that reduced or insufficient nutrition is the cause of the modification which occurs near the distal ends of the first feathers of birds.

The most highly modified region of the down, *i. e.*, the "quill" region, is produced in all cases so far as I am able to ascertain, after and soon after the hatching of the bird. There are the following reasons for a defective nutrition at that time:

after its formation further growth may be absolutely inhibited or stopped. If, therefore, faulty nutrition can and does completely halt the growth at the proximal part of the quill, there is every reason to believe that the same cause *may* have acted as the check upon the growth at the distal and all intermediate parts of the quill; and since it has been shown that the structures pro-

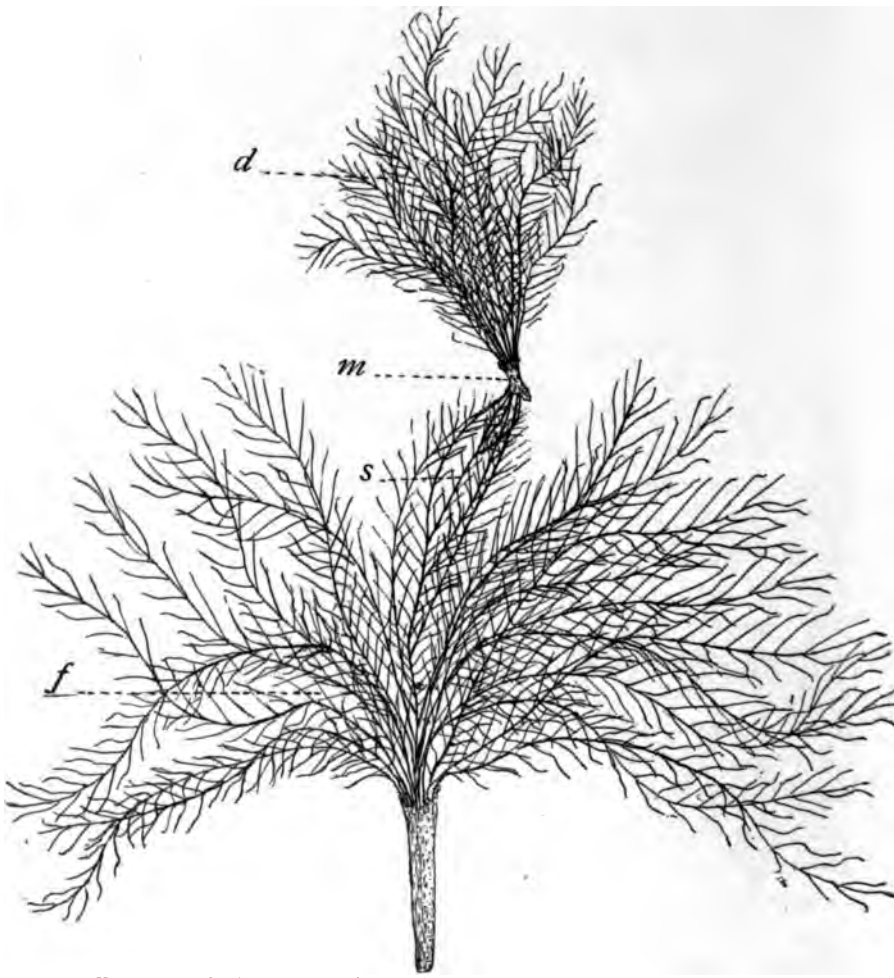

FIG. 1. A feather from the humeral tract of an underfed chick, four months old. *d*, downy portion; *m*, the highly modified basal region of the down, *i. e.*, the "quill." *s*, the barbule bearing shaft of the modified pennaceous feather *f* which grew under "starving" conditions and thus became downy or plumulaceous in character. Drawn with a camera lucida. Actual length of feather 3 cm.

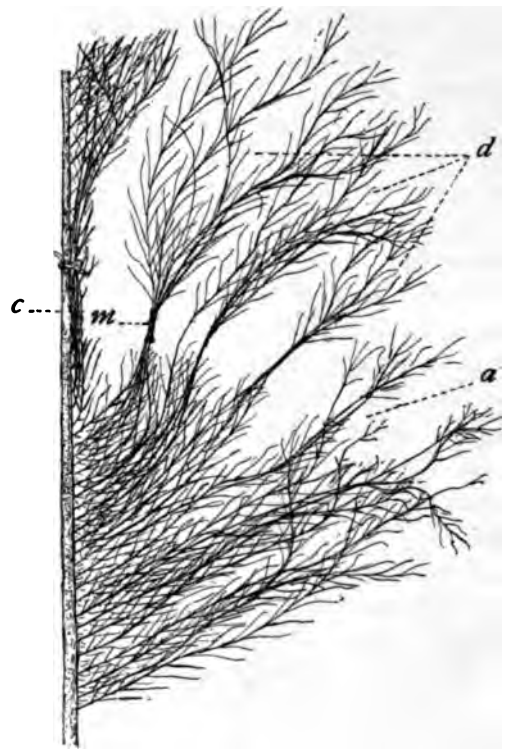

FIG. 2. A section of the proximal plumulaceous portion of a body covert from a chick, showing a modified region with "downy" formations *d*, the much-modified bases of which were produced during a "starving" period of two days. *m*, the horny covering (quill) of a bundle of six barbs; *a*, the abnormal area (fault-bar) produced at another point; *c*, the pigmentless part of the shaft which was grown during this period. Drawing made with camera lucida. Actual length of section shown 2 cm.

cell boundaries lost in a mass of fibrous tissue. Only the row of cells next to the pulp, representing the cylinder cell layer, retains its characteristic shape. At a still later stage in development, represented by Fig. 46, in which the epitrichial sheath is not shown, cornification of the outer rows of cells, representing the region of the sheath cells, has taken place, only suggestions of its original fibrous structure remaining. The outermost intermediate cells have become almost wholly fibrous, narrow spaces representing the position of the nuclei. The process of cornification now proceeds rapidly until practically all of the intermediate cells become cornified, and the cylinder cell layer becomes

say, many of the necessary cells never arise at all and those which are to be found in the barbule region never attain the normal size of such cells. Davies recognized, to a certain extent at least, a deficiency of growth of particular parts of this region. He states, "Gewöhnlich verschwinden (in the quill) die Leisten nicht vollkommen, obgleich sie eine bedeutende verminderung ihre Grösse erfahren" (p. 581). On the other hand, one kind of differentiation, *i. e.*, cornification or development of keratin proceeds without interruption in all of these cases.

Jones further states that apparently this "lack of differentiation . . . is induced by a reduced blood supply to this part of the feather, etc." He is here speaking of the conditions in the "quill"-formations only and does not apply this statement to the other form of down modification which he considers (p. 11) the typical one. He does not furnish any evidence for the statement just quoted, and does not refer to the direct and conclusive evidence which my paper ('07) supplies — and I may add that this is still the only direct evidence we have — that a reduced blood supply tends to produce just such feather modifications as are represented in the quill of the down.

Jones' observation that there appears to be no growth of the down in altricial birds during "this critical period" is important and suggestive as supporting the view that wherever we find the "down" we can assert that it signifies defective nutritive conditions in the bird at the time that part of the feather was grown. It is, however, doubtful whether Jones had anything similar to this in mind, for it will be seen that his last word on this subject is that "in the robin (the only specific case cited) the so-called 'quill' was formed at the proximal end of the down by the *rapid drying* of the imperfectly formed barb-vane ridges."

Finally, it should be noted that Jones states that in the case of the young robins the "quill" was formed *during* the fourth to the eighth days after hatching. It seems to me extremely probable that the first four days after hatching were even more important in producing the modification than the four succeeding ones.

TABLE I.

SHOWING RATE OF GROWTH OF RECTRIX OF RING-DOVE (*Turtur risorius*).

Days after appearance beyond skin.	1–4	5–8	9–11	12–15	16–20	21–25	26	27	28–32
Length of feather.	22	44	59	78	96	112	115	117	123
Average daily growth.	5½	5½	5	4⅘	4½ *	3⅗	3	2	1⅙

At the point indicated with the star (*), *i. e.*, at about 95 mm. from the distal tip of this feather, it is found that the plumulaceous formation begins. At first only those barbs which lie in the germ opposite to the shaft are affected; but as growth proceeds — and as the *rate of growth diminishes* — more and more barbs become affected.

After having watched the rate of growth of many feathers in chicks and doves only to find that the plumulaceous part always begins at the point of, or after, a considerable falling off in this rate of growth, one is tempted to the conclusion that in these feathers *the two kinds of feather growth, plumulaceous and pennaceous, are merely expressions of slow and rapid growth respectively.*

It is, I think, moreover, quite certain that for many birds the general rule can be laid down that those feathers which as a whole grow slowest have the greatest proportion of plumulaceous growth.

One is led by such considerations to inquire whether all strictly plumulaceous feathers are of slow growth. I know but little of these conditions from personal observation, but the known facts, in so far as I have been able to ascertain them, are in harmony with this view.

It is stated that the ostrich plumes grow at the rate of one inch per week. For such feathers of such birds this is indeed a slow rate — only about 3.5 mm. per day; whereas a little, newly hatched ring-dove will grow remiges and rectrices at from 5 to 7 mm. per day. I feel confident that it will be found that all plumulaceous feathers are grown at a relatively slow rate.

The aftershaft (hyporhachis) which is found in many feathers is another plumulaceous formation and like the others is of slow growth. It seems to me highly probable that a closer study of

the nutritive conditions in this region of feather-germs would reveal the reason for the presence of this feather-accessory in some plumes and its absence in others.

The true quill (calamus) also shares this slow growth of the proximal end of the feather. Indeed it is in the quill that we find the slowest rate of growth to be met with in the whole length of the feather. I am inclined to "explain" the quill as the type of feather formation which results from nutritive conditions which become slowly and progressively poorer; this in turn is able to almost completely stop growth and cell-division, but affects the process of cornification, *i. e.*, keratin formation, to a much smaller extent. It seems to me, too, that our knowledge of feather-growth (quill-formation) in the Japanese fowls, particularly the results of Cunningham's ('03) experiments, and many other facts support this conclusion. Neither the position nor the presence of a quill is "predetermined" in the feather, but both of these are merely marks left along the course of the ebbing tide of a greatly diminished feather nutrition.

The fact that plumulaceous structures do not show the maximum of growth and differentiation (*e. g.*, weaker barbs, and barbules without hooklets) together with the observation that such regions occasionally result from under-feeding (Fig. 1), would seem to lend weight to the view that such regions or such entire feathers are grown under nutritive conditions considerably below the optimum.

It should be remarked that if the view here put forward is correct it would lead us to expect a pretty general occurrence of growth-marks on all feathers which are growing at the time of hatching and soon thereafter. Such marks seem not to have been reported for the rather extraordinary first feathers of the Anserine birds. My own observations on this plumage of these birds are too meager to mention, but it seems quite probable that such marks are much less in evidence there — if they exist at all — than in most other birds. It is conceivable, however, that the young of these birds have a greater quantity of egg-yolk left for their first day or two after hatching; or that they have a considerable store of available fat in their bodies; or yet another means of tiding them over the "critical period" which in these

birds is of course very short. On this point, however, I can furnish no observations of value, and can only say that the exact conditions in these forms are not clear.

It must be left to the further investigations of those who in the course of their studies are able to examine the plumages (particularly the plumulaceous ones) of all of the families of birds to decide whether the theory here advanced of the slow rate of growth of the several plumulaceous formations can be universally and absolutely applied. It is the hope of the writer that some one may have the material and the inclination to put this part of the work here reported to a more rigorous test than the writer's limited material has permitted him to do.

Methods and Materials.

For the experimental part of the work here reported only a few forms have been used; but these few animals have been very closely watched and studied. Nearly all the starving experiments were made on the young of the chick (*Gallus domesticus*), and both on the young and old of the ring-doves (*Turtur risorius*). For the modifications of the nature of the feather growth the chicks are by far the better material. Control experiments were maintained throughout.

It should be said that the under-feeding or "starving" of these animals was usually either accompanied or accomplished by the feeding of the fat-stain Sudan III., which appears to "tie up" the fats of the body. The stain was fed for a purpose not immediately connected with the results reported here, but there is little doubt that it has no specific action on the feathers except in so far as it helps to bring about "starving" conditions in the animal.

Summary.

1. The highly modified region of the "down" is in all respects similar to other feather defects or fault-bars which have already been described as occurring at any and all levels in definitive feathers.

2. Juvenal feathers can by under-feeding be made to persist (chick) in the "downy" conditions practically without growth for several months if the reduced feeding be begun immediately after hatching.

3. Occasionally feathers may be found which have been able to continue their growth proximal to the downy portion despite the inhibitory influences of the lack of food; such feathers have been found to represent a type of structure intermediate to the downy and pennaceous formations.

4. The cause of the modification at the base of the down is to be traced to an interruption, or at any rate to the inadequacy of the nutritive processes of the bird. This interruption is doubtless partly accounted for by the change of source of food from the embryonic to the adult life.

5. The apparent absence of such growth-marks in the feathers of the duck and other anserine birds remains unexplained.

6. The rate of growth of the two ends of a juvenal, pennaceous feather, and of the proximal ends of all pennaceous feathers which bear a plumulaceous proximal portion, is much slower than the rate of growth in the central pennaceous part of the feather.

7. It seems probable that all plumulaceous structures are produced at a relatively slow rate of growth; and also probable that during their growth they have not enjoyed optimum nutritive conditions.

8. The formation of the quill is probably the direct result of a progressive diminution of an already lessened food-supply.

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THE LIFE HISTORY OF THE CARPENTER ANT.

JOHN LOSSEN PRICER.

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INTRODUCTION.

The studies reported in this paper were undertaken originally with the purpose of applying to another species of ants some of the tests and experiments which have yielded interesting results to other investigators, and incidentally I have been led to endeavor to work out the life history of a colony. In respect to the ecology of my species I have not aimed at completeness in any division, but have taken up whatever was at once most available under my conditions and most promising of results within the time at my disposal.

The work was done in the graduate school of the University of Illinois, as a part of the requirement for the degree of Master of Arts, and under the immediate supervision of Dr. S. A. Forbes, to whom I am deeply indebted for his many kindly and helpful suggestions and criticisms.

MATERIAL AND METHODS.

Two varieties of *Camponotus herculaneus* — *C. pennsylvanicus*, and *C. ferrugineus* — were made the basis of this work. So far as I have been able to learn, these varieties have exactly the same habits, the only difference noted being that *C. pennsylvanicus* is slightly more abundant in this region than the other variety.

The colonies used in my experiments were, for the most part,

collected from various small tracts of woodland within three or four miles of Urbana. These colonies were placed, after collection, in artificial nests of the Fielde type (BIOLOGICAL BULLETIN, Vol. II., No. 2), and were kept throughout the winter in a room of the insectory of the state entomologist, which is on the university campus. This room is heated by steam and was kept during the winter at a fairly constant temperature, ranging from 70° to 90° F. The ants were fed on sweetened water, pieces of insects, cooked lean meat, boiled eggs, etc., and all seemed to thrive perfectly on this fare.

My experiments were modeled after those of Fielde, Lubbock and others, but were usually modified in some details, in order to adapt them better to the species used; and a few experiments were specially devised to follow up and verify the conclusions arrived at. One outdoor colony was studied rather closely from about July 1, 1906, until their activities ceased with the approach of cold weather. All the work was done during the academic year of 1906–07, and all dates given in this paper are of this year.

LIFE HISTORY OF A COLONY.

Believing that a knowledge of the complete life history of such perennial colonies as are formed by the ants under consideration might throw considerable light on other important topics, I have undertaken to do what I could to work it out. The problem is a difficult one to handle in the short time of one collegiate year, and my results are necessarily incomplete.

My methods have been as follows:

1. I captured queens which had not settled in permanent quarters after their marriage flight, and placed them in artificial nests to rear their first season's broad of callows. I compared these small colonies as to number, size and general character of the individuals with many other similar colonies found in natural outdoor nests.

2. Throughout the winter I collected as many larger colonies as possible and carefully counted both the adult ants of all forms, and the larvæ. These colonies were collected when the temperature was low enough to make them inactive, so that by care-

September 22.....2	October 2.....1	October 15.....1
" 25.....1	" 5.....2	" 19.....1
" 27.....1	" 8.....2	" 20.....1

A few more eggs were laid during September, so that there were 14 small larvæ in the nest on October 15. These did not grow perceptibly until January, and then only slightly. During that month and at intervals afterwards, the queen laid a few eggs, and by May 1, the colony consisted of 15 callows, 21 larvæ and 8 eggs.

Taking the time required for the development of the first two callows as an approximate average, we have the following periods for the different stages: egg 24 days, larva 21 days, and pupa 21 days, making a total of 66 days from egg to adult. These periods are doubtless all liable to be affected by temperature and other varying conditions, for in outdoor nests some larvæ spend the winter in a state of arrested development, and I have kept one colony — No. 2, Table I. — in an artificial nest all winter in the insectory and no growth could be noticed in the larvæ until about March 1, when they suddenly began to grow at about the usual summer rate. On February 20 I gave five freshly laid eggs to a small colony with neither queen nor larvæ. Three of these hatched March 24, 28 and 30, respectively. The other two failed to hatch. These results show considerable variation in the length of the pupal period.

Another dealated queen of *C. pennsylvanicus* found on the sidewalk July 15 and placed in a nest, began laying eggs five days later, and continued at about the same rate as the one above mentioned, until twelve in all were laid. The first two of these eggs hatched August 13, an incubation period exactly the same as that of the first two eggs of the other queen. At this time the nest was allowed to become too dry and these two larvæ died and four of the eggs were destroyed. Four callows finally reached maturity, however, and the queen laid eggs at intervals throughout the winter, but was not very successful in bringing them to the adult form.

A little sweetened water was kept constantly in the nests of these two colonies and pieces of insects and some other forms of proteid food were occasionally given them, but from the time of

It is evident from the small range in the numbers of workers and larvæ, and from the similarity of these outdoor colonies in this respect to the two described above, that all the colonies rep-

FIG. 2. Same as Fig. 1, showing hole through which the queen found entrance to the cavity.

resented in the tables below were established during the summer of 1906. The difference in the number of individuals in the different colonies is probably due to the fact that the queens left their parental nests at different times, with different amounts of reserve food, and met with various vicissitudes in their efforts to

establish new colonies. Hence we may safely regard these as colonies of one season's development, or one year old colonies.

TABLE I.

SMALL COLONIES OF *C. pennsylvanicus*.

No.	Date.	Situation.	No. Queens.	No. Workers.	No. Larvæ.
1	Oct. 20	Decayed oak stump.	1	27	21
2	Nov. 3	Oak stump.	2	23	32
3	"	Cherry stump.	1	13	18
4	Dec. 28	Linden log.	1	3	12
5	Feb. 9	Ash stump.	1	15	18
6	"	Oak stump.	1	7	10
7	"	Cherry stump.	1	3	12
8	Feb. 16	Decayed oak log.	1	15	18
9	"	Oak stump.	1	22	30
10	"	Linden log.	1	8	18
11	"	" "	1	3	10
12	"	" "	1	4	19
13	"	" "	1	5	11
14	"	Oak stump.	1	11	20
15	"	Ash log.	1	6	9
16	"	Linden log.	1	2	15
17	"	Hickory stump.	1	4	18
18	"	Linden log.	1	7	25
19	Mar. 9	Hickory log.	1	18	32
20	"	" "	1	4	14
21	"	Hickory stump.	1	13	21
22	"	Oak stump.	1	16	17
23	"	Cherry stump.	1	3	8
24	"	Poplar log.	1	4	18
25	Mar. 23	Oak stump.	1	13	18
26	Apr. 6	Linden log.	1	4	12
27	"	" "	1	7	13
28	"	" "	1	21	20
29	"	" "	1	17	20
30	"	Oak stump.	1	9	10
31	"	Linden stump.	1	5	11
32	"	" "	1	9	8
33	Apr. 13	Linden log.	1	9	21
34	"	" "	1	7	13
35	"	" "	1	8	17
36	"	" "	1	3	14
37	"	" "	1	7	15
39	"	Hickory log.	1	19	22
40	Apr. 20	Linden log.	1	2	15
41	"	" "	1	24	30

For the remaining years of the life of a colony we shall have to depend on my second method, and this will give unsatisfactory results, especially because of the limited number of data which I have been able to collect. These data are presented in Tables III. and IV.

In addition to these data I have the following miscellaneous notes:

1. May 20, 1906, I caught a winged queen of *C. Pennsylvanicus*, crossing the sidewalk on the University campus.

2. June 12, 1906, I came upon a hollow tree with a small opening at the base. Around this opening were fifty or more male ants of *C. ferrugineus* in a state of great excitement, and the workers were dragging them back into the nest.

TABLE II.

SMALL COLONIES OF *C. ferrugineus*.

No.	Date.	Situation.	No. Queens.	No. Workers.	No. Larvæ.
1	Feb. 9	Oak stump.	1	15	25
2	"	" "	1	7	19
3	Feb. 16	" "	1	6	17
4	"	Hickory log.	1	9	15
5	"	Linden log.	1	10	21
6	"	" "	1	3	14
7	Mar. 9	Hickory log.	1	7	15
8	"	Oak stump.	1	12	26
9	Mar. 23	Hickory stump.	1	5	13
10	Apr. 6	Linden log.	1	7	12
11	"	" "	1	15	23
12	"	" "	1	4	10
13	"	" "	1	9	19
14	"	Linden stump.	1	11	21
15	Apr. 13	Linden log.	1	6	13
16	"	" "	1	5	11
17	Apr. 20	Cherry log.	1	13	26
18	"	Linden log.	1	2	16
19	"	" "	1	19	30

3. A friend told me of a colony of *C. pennsylvanicus* which inhabited a sill of his house and threw large quantities of particles of wood into his cellar. A large number of winged forms were seen about the outer opening of the nest about the first of July, and again about the middle of the same month.

4. July 6, 1906, I chopped in pieces a small decayed ash log and found a large colony of *C. pennsylvanicus* in it. Besides the workers there were probably 150 males, a large number of larvæ of all sizes, and approximately 200 pupæ, mostly of queens. About 50 workers, 30 pupæ, 20 larvæ and 15 males were taken and placed in a nest in the insectory. All the larvæ died before pupation, but seven queens and four workers emerged from the pupæ and lived through the winter in the nest. The queens emerged on the following dates:

July 25.....1	July 28 1	August 5.....1
" 27.....1	" 30.....2	" 7.....1

TABLE III.

LARGE COLONIES OF *C. pennsylvanicus.*

No.	Date.	Situation.	No. Workers.	No. Wingless Queens.	No. Winged Queens.	No. Males.	No. Larvæ.	No. *X. cava.*
1	Sep. 4	Apple tree.	2,500[1]	None seen	200	150	300	0
2	Apr. 13	Oak log.	3,018	1	196	174	842	27
3	Mar. 9	Oak tree.	2,609	1	207	116	486	4
4	Mar. 9	Oak log.	1,943	None found	104	102	235	3
5	Apr. 20	Linden log.	2,291	1	0	0	123	2
6	Feb. 9	Hickory tree.	2,139	1	0	0	867	0
7	Nov. 23	Boxelder tree.	1,872	1	0	0	216	7
8	Nov. 15	" "	1,246	1	0	0	196	2
9	Apr. 6	Linden log.	1,104	1	0	0	165	0
10	Apr. 20	" "	998	2	0	0	823	0
11	Apr. 13	" "	886	1	0	0	171	0
12	Apr. 6	Linden stump.	237	1	0	0	127	2
13	Apr. 13	Linden log.	167	1	0	0	74	0
14	Apr. 13	" "	139	1	0	0	74	0
15	Apr. 6	Linden stump.	122	1	0	0	106	0
16	Mar. 9	Oak log.	119	1	0	0	97	0

TABLE IV.

LARGE COLONIES OF *C. ferrugineus.*

No.	Date.	Situation.	No. Workers.	No. Wingless Queens.	No. Winged Queens.	No. Males.	No. Larvæ.	No. *X. cava.*
1	Dec. 28	Ash tree.	3,212	1	233	176	724	14
2	Apr. 13	Cherry log.	2,631	Not found	224	91	243	116
3	Apr. 20	Linden log.	2,214	1	131	97	322	51
4	Apr. 20	Oak log.	2,196	1	199	209	127	43
5	Mar. 23	" "	2,332	1	24	0	1,330	7
6	Apr. 6	Linden log.	327	1	0	470	75	22
7	Apr. 13	" "	143	1	0	0	87	7
8	May 4	" "	106	1	0	0	93	0

5. In the trees of the block in which I live are eleven colonies of *C. pennsylvanicus.* These colonies were observed almost daily after the first week of July until they ceased their activities with the approach of cold weather. Between July 18 and August 12 one of them, a very large colony, was seen daily to carry empty queen pupa cases from the nest. All the other ten colonies were watched carefully during this period, but only worker pupa cases were ever seen about them. The queens which were reared in

[1] The numbers in this colony are only estimated, as the ants were too active to permit of an accurate count.

the colony remained in the nest all winter, for I never saw any evidences of swarming, and two winged queens and one male were seen crawling about the nest on the evening of October 21.

6. On November 23 a friend who lives at Delavan, Illinois, discovered a large colony of *C. pennsylvanicus* which was living in a chest of small drawers which had been left undisturbed for three years in an old unused wood shed. This colony contained both males and winged queens.

From an inspection of Tables III. and IV. it will be seen, first, that with the exceptions of colony 6, Table IV., only the larger colonies contained the winged forms during the winter, and that all the largest colonies did contain them. In addition to this some of the larvæ of two of the large colonies which did not contain winged forms, viz., colonies 6 and 7, Table III., and which were kept in nests in the insectory after capture, proved to be male larvæ, and in both these colonies, moreover, the workers laid many eggs during the winter. As the queens of both colonies died soon after capture, the eggs that appeared in the nest must have been those of workers. It has been fairly well established that the eggs of workers usually develop into males, and hence one may be certain that these two colonies would have produced males during the summer of 1907 had they been left undisturbed; and since, as the tables show, winged forms of both sexes usually occur at the same time, it is quite probable that they would have produced queens.

Putting together the foregoing observations we may draw some more or less definite conclusions.

1. In two observed instances queens appeared in the adult form during the latter part of July and the first part of August, and in one of these instances these queens emerging at this time remained in the parental outdoor nest over winter.

2. One winged queen of *C. pennsylvanicus* was observed out of the parental nest on May 20; a colony of *C. ferrugineus* was seen in the act of swarming on June 12; and a colony of *C. pennsylvanicus* was reported to have swarmed during July.

3. All of the colonies of Tables I. and II. must have been established at least as early as July.

4. Nearly all the larger colonies were found to contain winged forms of both sexes during the winter.

5. None of the ten colonies of intermediate size which were observed closely during July and August were seen to carry queen pupa cases from the nest, and only one colony of this type represented in Tables III. and IV. contained winged forms during the winter.

These facts make reasonably evident the following conclusions:

First, that a colony does not produce winged forms until it is more than two years old.

Second, that a brood of winged forms is produced during one summer, remains in the parental nest over winter, and leaves for the marriage flight during a time ranging from May to July.

In regard to the number of years required for a colony to reach sufficient maturity to produce sexually perfect individuals I have the following data:

1. The two queens which reared their first young in artificial nests laid eggs at the rate of about two a day, during the regular season, and several others taken during the winter with small colonies have laid eggs at about the same rate part of the time since being brought into the insectory.

2. In the sixty colonies of Tables I. and II. the largest number of workers in any one colony is twenty-seven, and the largest number of larvæ is thirty-two.

3. In Tables III. and IV. we have the following rather distinct groups of colonies as regard size:

(*a*) Eight, with the number of workers ranging from one hundred and six to two hundred and thirty-seven, and the number of larvæ from seventy-four to one hundred and twenty-seven.

(*b*) Four, with the number of workers ranging from eight hundred and eighty-six to twelve hundred and forty-six, and the number of larvæ from one hundred and sixty-five to eight hundred and twenty-three. The colony with the largest number of larvæ, however, possessed two queens.

(*c*) Three, with the number of workers ranging from eighteen hundred and seventy-two to twenty-two hundred and ninety-one, and the number of larvæ from one hundred and twenty three to eight hundred and sixty-seven.

(*d*) Nine, with winged forms and with the number of workers ranging from nineteen hundred and forty-three to thirty-two hundred and twelve.

4. As I shall show in more detail later under polymorphism, the workers which a queen produces the first season are all of the very smallest size and, as the colony increases in size, larger and larger workers are produced until, in colonies of the size in group (*b*) above, a few of the largest size appear.

5. As I shall show later, under division of labor, these largest sized workers seem to take no part in the work of gathering food for the colony, but remain in the nest and seem to possess largely the instincts of queens.

6. Colonies 6 and 7, Table III. and colony 1, Table IV., were kept in artificial nests in the insectory after capture, and the workers laid eggs abundantly during the winter and a large number of these eggs developed into males.

7. Two smaller ones, 8 and 16, of Table III., were also kept in the insectory after capture, without queens, and were fed just the same as those mentioned above and no eggs were seen with either of these.

These data make reasonably evident the following conclusions:

1. Sexually perfect individuals are not produced until the colony consists of approximately two thousand workers, and they are produced by nearly all colonies of this size or larger.

2. From three to six years or longer are required for a colony to reach this size.

The fact that neither eggs nor pupæ are found in the nest during the winter, and that the larvæ are all very small, must mean that the proper feeding of the young larvæ and the egg laying cease several weeks before the temperature is too low for the process of incubation. This is supported by the fact that colony 1, Table III., which was taken on September 4, contained neither eggs nor pupæ, and only very small larvæ. The cessation of these two processes is probably caused by the workers and queens storing up food in their own bodies for the processes of metabolism during hibernation. If so, egg laying and the feeding of the youngest larvæ probably cease at about the same time, and the winter larvæ are hatched from the eggs which are in the nest when the queen stops laying. Hence we have in the number of winter larvæ an indefinite clue to the rate of egg laying in colonies of different sizes. The average number of larvæ in the

are produced. That in this way the life of a colony may come to a natural end, is supported by the following observations:

1. On June 12, 1906, I came upon a large oak tree which was hollow at the base, and was inhabited by a colony of *C. ferrugineus*. Fifty or more males were seen about the opening of the nest in a state of great excitement and the workers were dragging them back into the nest. About a month later I revisited the tree and not a single ant was seen about the place, although I watched it for about two hours. Twice afterwards I visited the tree with the same result.

2. November 3, 1906, I tore to pieces a large oak stump which was so badly decayed that I could break it to pieces with my hands. It was thoroughly riddled by the work of insects of various kinds, and showed plainly that it had recently been inhabited by a colony of *C. pennsylvanicus*, for besides the characteristic appearance of the galleries, I found fragments of workers' bodies, a few pieces of the wings of queens, and five live males and three large workers. Plainly a colony had recently moved from the place or had there reached the natural end of its life.

3. Colony 6, Table IV., with its 327 workers and 475 males, was very likely a degenerate colony which had about reached the end of its life. This colony was found in an old linden log which was so badly decayed and riddled by galleries which had evidently at different times been occupied by the colony, that it was just about to fall in pieces, and the whole scene presented every appearance of age. As is shown in Table VI., under "polymorphism," this colony contained a comparatively very large per cent. of the largest sized workers and a very small per cent. of the smallest sized workers, and this also is an indication that the colony had existed longer than the natural life-time of the small-sized workers which are produced in such a large proportion the first two years. The large-sized workers were produced later in the life of the colony and hence we might expect to have a larger per cent. of them in a degenerate colony.

Polymorphism.

The principal value of a knowledge of the complete life-history of a colony is, I believe, in the light which it will throw on the

problem of polymorphism among ants in general a problem on which much has been done and much written, but which I think, has not hitherto been examined from this standpoint.

The form of polymorphism we find here is what Eschreich in his "Die Ameise" calls incomplete polymorphism. That is, there is no distinct soldier-type, and there is a regular gradation in the size of the workers from the very largest to the smallest,

FIG. 3. Workers of *C. pennsylvanicus* arranged according to size and division of labor.

as shown in Fig. 3. I have grouped the twelve sizes shown in this figure into four subgroups to which I shall refer as Nos. 1, 2, 3 and 4, as indicated in the figure. This division into groups, I shall attempt to show later, is in harmony with an incomplete division of labor that exists among the workers.

The point revealed by a study of the life-history of a colony that I think is of importance is the fact that these different sizes of workers, and finally the winged forms, are produced at rather definite periods during the life of the colony. This is shown in Tables V. and VI.

The colonies represented in these tables were all killed in cyanide bottles and were then divided as accurately as possible into groups according to the four sizes represented in Fig. 3.

In all the colonies represented in Tables I. and II. every one of the workers was of size No. 4, and in the smaller colonies of Tables V. and VI., which, according to our previous conclusion, were two years old, this size still very largely predominated. In this latter group of colonies were found, however, a small number of size No. 3, and a still smaller number of No. 2, but not a single No. 1. From this point on, so far as the tables go, all sizes were found in all the colonies, and with the exception of colony 6, Table IV., not any perceptible increase is shown in the per cent. of the largest sized workers as the colony increased in

size, yet this per cent. varied within a narrow range for the different colonies. The fact that none of the largest size appeared in the one and two year old colonies cannot be merely accidental, for if all these colonies represented in the tables were taken together they would form a large colony, and yet not a single individual of size No. 1 would be found among them, while in the largest colony of Table V. there were 77 of this size.

TABLE V.

C. pennsylvanicus.

No. of Colony in Table III.	Workers Size No. 1.		Workers Size No. 2.		Workers Size No. 3.		Workers Size No. 4.		Total No. of Workers.	No of Larvæ.	No. Winged Queens.	No. Males.
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.				
15	0	0	11	9.0	17	13.9	94	77	122	106	0	0
14	0	0	2	1.4	11	7.9	126	90.6	139	74	0	0
13	0	0	5	3.0	27	16.4	132	80.5	164	74	0	0
11	21	2.4	80	9.0	212	23.9	573	64.7	886	171	0	0
10	19	1.9	123	12.3	313	31.4	541	54.2	998	823	0	0
5	26	1.2	127	5.5	478	20.9	1,660	72.4	2,291	123	0	0
2	77	2.5	575	19.0	1,385	45.8	981	32.5	3,018	1,042	196	174
						C. ferrugineus.						
8	0	0	7	6.7	28	26.4	71	66.9	106	93	0	0
6	23	7.0	121	37.0	163	49.0	20	6.1	327	75	0	475
4	42	1.9	301	13.7	997	45.4	855	38.9	2,196	127	199	209
3	53	2.4	298	13.4	971	43.8	892	40.3	2,214	322	131	97
2	74	2.8	337	12.8	1,063	40.4	1,142	43.8	2,631	243	224	91

We may then consider it fairly well established that none of the largest workers are produced during the first two years of the colony's life, and that the sexually perfect forms are not produced until the colony is at least four or more years old. If it is true that the male forms usually arise from eggs laid by workers, we may add to the above that egg-laying workers do not appear until just preceding the production of winged queens. We then have a rather gradual increase in perfection from the smallest worker produced by the queen the season of her marriage flight to larger workers, then to egg-laying workers and finally to perfect winged females, and if the queen lives throughout this period, and it is altogether likely that she does, the eggs that produce these various forms are all laid by the same queen. An expla-

nation of this is not easy, yet I presume that I shall do no violence if I attempt one.

The most obvious external condition which may be responsible for this phenomenon is the food supply of the colony. During the first years the workers are few and the domestic duties are proportionally large. The permanent home must be established and there is consequently comparatively little time for food gathering, and probably in many cases relations are not readily established with a suitable herd of aphids, so that in all probability the larvæ of these years are scantily fed or are fed a less varied and less concentrated food than that given those that appear when the colony is more mature. During the later years the formicary is well established and there are larger and more powerful workers to make what extensions are necessary. The working force is increased proportionally more than the number of larvæ to be fed. These workers range over a wider field and collect not only more, but a greater variety of food. There can thus be little question that the larvæ of the large colony are better fed than are those of the small one, and since the better feeding is parallel with the production of more perfect forms, it seems only reasonable to believe that there is some relation between them. If it be asked, why do not all the workers of a given season develop into the same size or form, I think that I can say in reply that the food is not equally distributed. I have had numerous instances of a few of the larvæ developing much more rapidly than others in my artificial nests. The winter larvæ when taken were all practically of the same size, and many of them remained unchanged in size for several weeks after being brought into the insectory, while others, usually a small portion of the whole number, soon began to grow quite rapidly.

This leads us to the conclusion that the variations in form are ontogenetic in origin, that the fertilized eggs of the queen are all essentially alike when laid, and each capable of developing into a small worker or a winged queen. This conclusion is supported by a view proposed by Emery in a paper entitled "Die Entstehung und Ausbildung des Arbeiterstandes bei den Ameisen." I quote the following sentences of this paper from Wheeler's "Polymorphism of Ants" (*Bulletin of American Museum of Natural History*, Vol. XXIII., Article I.):

"The peculiarities in which the workers differ from the corresponding sexual forms are, therefore, not innate or blastogenic, but acquired, that is somatogenic. Nor are they transmitted as such, but in the form of a peculiarity of the germ plasm, that enables this substance to take different developmental paths during ontogeny."

Wheeler remarks in connection with this quotation that the view presented has never received the attention that it merits, and I trust that the data that I have brought out in connection with the life-history of a colony may serve to strengthen it appreciably. Wheeler has also elsewhere ("A Neglected Factor in Evolution," *Science*, N. S., Vol. XV., pp. 766–774) referred to the influence of the age and trophic status of the colony on the variability of the polymorphic ants.

Division of Labor.

Division of labor among the workers, like their polymorphism, is incomplete; and yet, in the one outdoor colony which I studied, very marked traces of it were seen. This colony, which I shall designate as colony A, lived in a large maple tree which stood on the border of a city block containing but three houses, the rest of the block being vacant, and allowed to grow up in weeds. One hundred and fifty feet away from the nest tree of this colony and just at the rear of one of the houses, stood a cottonwood tree, five or six years old; and near this was a clump of small boxelder trees. The cottonwood was infested with one species of aphis and the boxelders with another. The ants adopted these two "herds" of aphids as their main source of food, but showed a decided preference for those on the cottonwood. At the base of this tree they constructed a temporary chamber, by entering into a crack in the ground and carrying out the particles of earth as they do the particles of wood from their permanent home. After they had used this retreat for a time, I tore it open and, by means of glass plates, constructed a chamber for them somewhat like a Fielde nest, covering it with a piece of orange-colored glass through which I could easily observe what occurred beneath. This the ants readily accepted as equivalent to the one that they had constructed, and used it throughout the summer.

The first aphid appeared on the tree about May 1, 1906, and when I first noticed it a single ant was attending it. Gradually, as the colony of aphids increased in numbers and spread over the tree, the number of ants to be seen there also increased proportionally. My attention was very early called to the fact that, although some of the larger workers (size no. 2, Fig. 3) were often about the base of the tree, they never ascended to the aphids. The work of attending the aphids was performed entirely by workers of size no. 3. After the aphids became abundant I repeatedly saw ants of this size coming from the hole in the ground and ascending the tree to the aphids, and later returning. At the same time numerous workers of size no. 2 were coming from the nest and entering the hole in the ground, and others of this size were leaving this hole for the nest. On the evening of July 20, I made an observation which explained these actions. A no. 3 came down the tree and before entering the ground was accosted by a no. 2 and responded by giving up to her larger sister apparently all that she had gathered above. While their mandibles were interlocked in the process of transferring food the abdomen of the smaller one kept up a constant quivering, jerking motion, seemingly in an effort to regurgitate the last drop of food in her body. After this was over the smaller ones returned to the aphids, and the larger one entered the apartment in the ground as if not yet satisfied to return to the rest. This led me to construct the artificial chamber mentioned above and in this I have seen this process repeated many times. I find, however, that it is seldom that the no. 3 gives all the food that she has to offer to the first no. 2 that approaches her. During the daytime when only very few of the ants were active, a large number of the smaller workers were at rest in the quarters I had constructed for them and not much exchange of food was going on, but when I saw them here just after they had begun their evening's activities, or examined this chamber by means of a light, after dark, as many as two thirds of those in the chamber were paired off in the act of exchanging food. At these times, between the aphid tree and the nest was a caravan of workers going and coming, and these were very largely, though not wholly, of size no. 2. Those returning to the nest had their abdomens distended

until they appeared, at first glance, much larger than those traveling in the opposite direction. Thus, so far as this one colony is concerned, the food was gathered almost entirely by workers of these two intermediate sizes; the one, no. 3, so far as I have observed, without exception, first gathering the fluid from the aphids, and no. 2 principally transporting it to the nest, though aided by some of no. 3.

I have not observed other colonies sufficiently to determine whether this practice is general among them, though it surely cannot be universal for many colonies find their aphids on the same tree in which they live. This was a mature colony of large size and had probably perfected this division of labor gradually as the colony developed in conformity to the conditions that surrounded them.

The work of the two extreme sizes is more difficult to make out, for they are in some way the "house-keepers," that is, they are, during the daytime, at least, about the nest. The smaller ones, size no. 4, are often seen carrying the particles of wood and the empty pupa cases from the nest. Taking into account the immense mandibles of size no. 1, one might suppose that these are the true carpenter ants, and that it is their business to build additions to the formicary; but as has been shown in the tables above, they appear in the colony after it is pretty well established, and then occur in rather small numbers we must at least conclude that they are not the only carpenters in the colony. In general behavior they resemble very much the virgin queens. In the artificial nest they remain constantly in the dark room, and when the colored glass is removed they are among the first to seek shelter and the last to show fight. I have had since capture colony 6, Table III., in a nest which is connected with a feeding-room by means of four glass tubes each about three and a half feet long, three of them coiled and one straight, and I have never seen a no. 1, in the feeding-room, although there are fifty of them in the colony. The queen of this colony died soon after capture, and yet many eggs have been laid, I consequently feel quite certain that the no. 1's are principally egg-layers, and it is probable that along with the development of the ovaries some of the instincts of the queen also appear, and a corresponding lack of certain other instincts possessed by the common workers.

that some special skill, not possessed by the foraging worker, is required to extract it.

I have frequently seen individuals of *C. pennsylvanicus* feeding on an apple, and on one occasion saw them extract the juice from a large stalk of pie-plant. This material was available to them for some time during the season, but they helped themselves to it only once. I once saw a colony which lived in the trunk of a large ash tree feeding on the pulp of a water-spout of the tree. They had removed almost every particle of material from within the bark for a distance of about a foot, so weakening the sprout that it had bent over. This had been done within a short time, for the leaves above the injury, although wilted, were still green, and a few of the ants were yet working on it when I saw them.

These ants seem to possess great power of husbanding the nutriment within their own bodies. I have kept colony 1, Table IV., in the insectory since January, giving them no food but sugar and water, and yet they have successfully brought to maturity all or nearly all their larvæ, their workers have laid many eggs, and the colony is now, May 10, to all appearances, as healthy as any under my care. The proteid food required for the feeding of the larvæ and for maturing the eggs must have been in store in some form in the bodies of the workers. I have also noticed, with respect to the colonies which I have collected since the few warm days we had in March, that many of them are much larger than any I saw in outdoor nests previous to that time. Very few of these ants have even yet, May 10, been seen out of the nest, and the food upon which the larvæ have grown must have been a surplus of that stored for the purposes of respiration during the winter. I have two colonies, viz., 16 and 12, of Table III., to which I have given no food since April 6. Colony 16 had been given the usual indoor fare since capture up to the time mentioned above, and colony 12 was captured on the day the experiment began. Both colonies are now, May 10, apparently as healthy as any others that I have in confinement.

This faculty adapts them admirably to the conditions of their life, for gathering their food as they do, and being unable to store it otherwise than in their bodies, there is likely to be consider-

able variation in its character, and considerable fluctuation in its amount.

Relations to Light and Color.

In endeavoring to work out the relations of these ants to light and color I have resorted to experiments modeled after those made by Fielde, Lubbock and others, on other species of ants. I have, however, used slightly different apparatus from that used by either of these investigators. In the first place I constructed a nest of the Fielde type which was twenty-four inches long and nine and one half inches wide, and which contained a hall-way one and one half inches wide, running longitudinally through the center, with six rooms, each 4 by 4 inches, on either side of the hall-way. The outer walls of the nest were bound by black binding paper and the walls between the rooms were made of two pieces of glass with a strip of black paper between them, so that all the walls of each room were perfectly darkened and no light could enter the rooms except through the glass plates placed over them and through the small pieces of glass tubing which formed the entrances from the hall-way. The nest was connected with a feeding-room by means of a piece of glass tubing which led from one end of the hall-way. The hall-way was covered with a strip of clear glass, and as covers for the rooms I used glass plates of the following descriptions:

1. A deep red glass which transmitted only the red rays of the spectrum.

2. A brownish orange glass which transmitted all of the red end of the spectrum including a large part of the green.

3. A green glass which transmitted all of the green rays and a small part of the red.

4. A deep blue glass which transmitted all of the blue end of the spectrum, including a very little of the green.

5. An indigo-blue glass transmitting all colors of the spectrum to some extent, but showing narrow absorption bands in the red and green.

I also used cells containing carbon disulphide to shut out the ultraviolet rays.

With this apparatus I performed the following experiments:

Experiment I.

January 1, 1907. — On one side of the hall glasses were placed on the rooms in the following order: red, orange, green, indigo, blue, clear; and on the other side in the reverse order, so that the two red glasses were on diagonally opposite corners of the nest. A colony of *C. pennsylvanicus* was introduced into the feeding-room. This colony was just large enough to fill comfortably two of the rooms, and too large to get into one.

January 2. — All ants were settled in the two rooms covered by red glass. The red plates were now exchanged with the two clear plates, and on January 3, all ants were again collected under the red glass. These red plates were now removed from the nest and were replaced by plates of clear glass. On January 4, about one third of the colony were in one room under green glass, another third were under orange, and the remaining third stayed where they were under clear glass for four days, finally joining their companions under the green and orange respectively on January 8. These glasses were now exchanged with the two indigoes, and the ants remained unsettled for a whole week of dark, cloudy weather, as many of them remaining under clear glass as under any other. On January 15, I placed double thicknesses of orange glass over two of the rooms, and on January 18 all ants were collected in these two rooms. I now removed the orange glasses from the nest, replacing them with clear glass, and on January 19, after a few hours of bright sunshine, the ants were all collected under the two green glasses. These were then exchanged with clear glass and the ants were again unsettled for a period of six days, when a bright day caused them to settle under the green glasses on January 25. These glasses were now removed from the nest and replaced by clear glass. The nest was then left for twelve days in this condition with only the blue, indigo and clear glass over the rooms, and although there were several bright days during the time, the ants never settled in any one room, but seemed to be endeavoring to escape. On February 6 I replaced two of the clear plates with the two green plates, and on February 8 all ants were collected in these rooms. I then placed the orange plates back on the nest, but no ants collected under them. On February 15 I

placed the red plates back on the nest and the following day about twenty ants were collected under one of them, but not all of the ants removed to the red glass, however, until February 22. The glasses were now arranged as at the beginning of the experiment and the carbon disulphide cells were placed over the two clear plates. The experiment now proceeded as before, with no essential difference in results, until the red, orange and green glasses had been removed from the nest. The green plates were removed on March 8, and two days later all ants were collected under the two disulphide cells. I then returned the green plates to the nest, and the following day ten ants were in the room under one of them. The number that left the disulphide cells for the green gradually grew until, on March 17, all had done so.

Experiment II.

This colony was now removed from the nest and, after the latter was thoroughly cleaned, another colony of *C. pennsylvanicus* containing a large number of winged queens and males was introduced. The glass plates were again arranged as at the beginning of the experiment and the disulphide cells were placed over the clear plates. The colony would have filled about three of the rooms, but they scattered out and occupied eight of them, omitting entirely, however, the two indigoes and the two blues. The nest was left as first arranged from March 20 to May 12, and some ants were seen at all times in each of the eight rooms in which they first settled, except a few days while the disulphide cells were removed. During all this time only occasional stragglers were ever seen under the blue or the indigo plates.

Experiment III.

A number of the queens of the colony used in experiment II. were removed for other experiments and the colony was reduced in size until it could easily occupy two of the rooms, and then, on May 12, the nest was taken into a room which admitted no light from the outside and which was supplied with an arc light of four hundred and eighty candle power. The nest was placed about three feet below the arc light, and a little to one side so as to avoid the shadows of the lamp. The glass plates were

arranged as in experiment I., and the disulphide cells were placed over the clear plates. The ants immediately collected under the red plates after the arc light was turned on. These plates were then exchanged with the two blue plates, and twenty-five minutes later the ants were again under the red plates. These plates were then replaced by clear plates, and thirty minutes later all ants were collected under the two orange and one of the green plates. The orange plates were again exchanged with the blue, and in twenty-five minutes the ants were under the two green and one of the orange plates. The green plates were then removed from the nest and thirty-five minutes later all ants were under the two orange plates. These were then removed from the nest and forty minutes later all ants were under the two disulphide cells. The two disulphide cells were now placed over the blue plates and in twenty-five minutes all ants were collected under them. Next the cells were moved to the indigo plates and in thirty-five minutes all ants were again under them. The cells were then placed back on the clear plates and in thirty-five minutes the ants were again under them. The red plates were then placed back on the nest, and two hours later some of the ants were still under the disulphide cells, although most of them had moved to the red plates.

These results indicate plainly that, when forced to choose between light of different wave-lengths, these ants have a decided preference for the red or longer rays and a decided dislike for the ultraviolet rays. The last part of experiment III. also indicates that they prefer the red rays to the blue and violet rays. In these respects these ants seem to agree perfectly with the ones which Lubbock experimented upon ("Ants, Bees and Wasps," pp. 211 to 217) and also with those of Miss Fielde ("Notes on An Ant," Philadelphia Academy of Science, Vol. 54, pp. 614 to 625).

Experiment IV.

Twenty-five workers of *C. ferrugineus* were cooled until they were inactive and then their eyes were carefully painted with a mixture of liquid glue and lamp black. They were then placed in a Fielde nest consisting of two rooms, four by four inches, which were joined by a narrow passage-way. One room was

disappeared from sight. During the first eighteen minutes I counted only twenty-two ants, and then, as if by a sudden signal, the procession began to move, and during the following eighteen minutes I counted five hundred and twenty-two ants, and about this time the supply in the nest below seemed to be almost exhausted. A little before I stopped counting, large numbers of the larger workers began to arrive from the nest tree and to enter the temporary nest at the base of the tree. I have observed this colony repeatedly at different times during the night, and as late as two o'clock in the morning, and have always found them very active.

Architecture and Economic Relations.

McCook describes in detail the architecture of a colony of carpenter ants which he found inhabiting a corner beam of an old mill. ("A Guild of Carpenter Ants," *Harper's Monthly*, July, 1906.) In the same article he discusses serious injuries to forestry and lumber interest which have been reported to have been done by the carpenter ants, and he also reports railroad accidents which were thought to have been caused by carpenter ants weakening the timbers of bridges.

Dr. E. P. Felt, state entomologist of New York, also accuses the large, black carpenter ants of doing much injury to forests. ("Insects Affecting Forest Trees," Seventh Report, New York State Commission of Forest, Fish and Game, p. 522.) In this

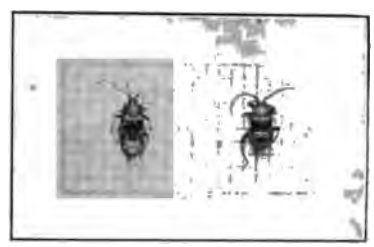

Fig. 4. Two specimens of *Xenodusa cava*.

article he shows in cuts the very different styles of architecture followed by the ants in elm and in balsam and supposes that the difference is due to the different structures of the two woods.

The observations which I have made while collecting the colonies represented in the tables of this paper lead me to believe

the voided wood that the larvæ had left in the burrows. As the wood gradually decays and in cases where the ants follow larvæ that bore in decayed wood they do enlarge the galleries and shape them into chambers which are more or less characteristic, but even here the style of the architecture is determined largely by structure of the wood and the instinct of the particular larva followed. The influence of the structure of the wood is shown in Figs. 5 and 6. Fig. 5 shows a radial section of decayed oak in which the more durable medulary rays formed a limit to the gallery in one direction and in Fig. 6 is shown a tangential section of the same wood in which the dense summer wood of an annual ring forms the limit in the other direction. Many observations similar to these have convinced me that these ants do not build their own galleries in solid wood. They either follow the wood-bores or work in badly decayed wood and if this conclusion is true their economic importance must be extremely slight.

Guests and Parasites.

Probably the most distinguished and interesting guests which have been found with ant colonies are certain Lomechusini, several species of which are very common guests with the ants of continental Europe. This family is represented in North America by the single genus *Xenodusa*, and the best known representative of the genus is *X. cava*, Fig. 7. Wheeler, in a review of the observations made on this beetle ("Polymorphism of Ants," *Bulletin American Museum of Natural History*, Vol. XXIII., pp. 35–40), shows that, so far as reported, only five persons, viz.: Leconte, Blanchard, Muckermann, Schwarz and himself, have ever seen it and each of these only rarely. Schwarz found it with *C. pennsylvanicus*, and Blanchard with a colony of large black ants which were probably of the same species. No one has before seen it with *C. ferrugineus*. By referring to Tables III. and IV. of this paper, it will be seen that I have found it to be quite abundant in this region, and by a comparison of these two tables, it will be seen that the beetle seems to prefer *C. ferrugineus* as a winter host. Wassmann and Wheeler are of the opinion that the beetle simply hibernates with these larger ants and then in the spring migrates to the nests of some smaller ants

as a summer host. In fact Wheeler found, July 1, 1905, six larvæ of *X. cava* in a nest of *Formica incerta*, and since the European relatives of the beetle are found mainly with this genus

FIG. 7. Galleries in solid ash wood which were inhabited by *C. ferrugineus*.

of ants, it seems quite probable that the summer host of *X. cava* may be some one or more of the *Formicas*. During the month of June, 1907, it was my hope to discover the summer host and I accordingly opened a good many nests of *Formica* in the vicinity of the woods in which I found the beetle most abundant, but was unable to find a trace of either adult beetle or of larvæ. I did, however, find adult beetles in the nests of *C. ferrugineus* at differ-

ent times during the month. On June 10, I opened two nests seeing five beetles in one and three in the other; on June 20, I found four beetles in a nest of these ants and on June 30, I opened a nest and saw two of the beetles. These may have been a few stragglers which had not migrated because of some disability yet I could not see any signs of imperfection in them. Since Wheeler found larvæ on July 1, it seems quite probable that at least some of the beetles spend the summer also with the winter host.

The beetles are always royally received into the nests of the two varieties of ants that I have been studying. I have kept them all winter in artificial nests with the ants, have transferred them from one colony to another and have placed them in the nest with colonies which had none in their out-door nests, and they were always received and cared for. I have repeatedly seen the ants licking them and feeding them, and when the beetles strayed out to the feeding-room of the nest the ants would pick them up and carry them back to the nest.

Another guest or parasite which I have found abundantly with the colonies of *C. pennsylvanicus* is a small red mite, which, according to Nathan Banks, to whom I sent some mounted specimens, is an undescribed species of *Uropoda*. I have not been able to determine whether these are parasitic or merely attached to the ants for the purpose of transportation, but am inclined to think the former, as was also Mr. Banks. They were always attached to the ants at the joints of the legs, or on the underside of the joint between the head and thorax and were probably extracting their nourishment through the thin nonchitinous membranes of these regions. It seems a little peculiar that the ants permitted them to stay there, for evidently they could easily have removed them. It is possible, however, that the mites secured this attachment after the ants became inactive from the cold, for I collected them all during the winter and noticed that the mites disappeared within two or three days after being brought into the insectory.

Besides these I have found numerous other insects which live either in the nest or in very close proximity to it and seem to live peaceably with the ants. Among these are various staphy-

linid beetles, several species of Blattidæ, and the adults of the wood-boring larvæ. Other ants are also often very close neighbors of the species I have studied. I have found two colonies of small ants in chambers which were connected on all sides with those occupied by the larger ants. One of these was a colony of *Myrmecina americana*, which I found with a colony of *C. ferrugineus*, and the other a colony of *Monomorium pharaonis*, which I found with a colony of *C. pennsylvanicus*. The insectory is badly infested with this latter species and they have helped themselves liberally to the food that I have given my ants, and have especially thrived on the dead ants which my colonies threw upon their rubbish heaps. These little ants are not noticed by the larger ones, and doubtless in the natural nests they subsist largely by picking up the "crumbs" about the formicaries of the larger species.

Instincts and Intelligence.

The many remarkable feats performed by ants in the round of their daily life have led observers to form various conclusions as to the parts played by instincts and intelligence respectively, in controlling their movements. Lubbock, on the one hand, concludes that ants rank next to man in the degree of intelligence possessed ("Ants, Bees and Wasps," p. 1), and on the other hand, Wassmann endeavors to show that they are absolutely void of pure intelligence ("Psychology of Ants").

On this subject I have made the following observations and experiments:

Observation 1.— On the evening of August 1, 1906, I planned to count the number of workers of outdoor colony *A*, which passed a certain point between the nest and the aphid tree, in a given time. In order to do so I cleared away the weeds so that I might see them more easily. I soon noticed, however, that ants were collecting on both sides of the place where I had disturbed the path, and they refused to cross the disturbed area. Considerable excitement seemed to prevail on both sides, and the number kept increasing, especially on the side toward the nest. Some of these returned to the nest and some of the others returned to the aphid tree. Finally, after about thirty minutes, when approximately one hundred ants were assembled on the

side toward the nest, this group began to advance slowly into the disturbed territory. After much retracing of steps they succeeded in about three minutes in crossing this area, which was only about one foot in diameter, and they then hurried on their way unhesitatingly.

Observation 2.— About fifty feet of the path of this colony from the nest to the aphid tree lay through a dense weed patch. Early in the summer I had smoothed out with a hoe a narrow path in a fairly direct line from the nest to the aphid tree, and by repeatedly passing along this path myself, I kept it worn smooth all summer. I repeatedly observed that the ant caravan used this path only at points where it happened to coincide with a perfectly direct line between the point at which they entered the weed patch and the point at which they emerged from it.

On the afternoon of August 4, 1906, some men came with a plow and tried to plow the weeds under. The weeds were three or four feet high, and when the plowing was finished the lot seemed to me an impassable barrier to the movement of the ants. That evening, as the ants began to pour forth from the nest for their night's work, great excitement ensued when they reached the edge of the plowed ground, which was about five feet from the nest. Soon a space about one foot wide and reaching from the nest to the plowed ground was literally black with ants, all running back and forth and behaving very much as people do in case of a fire in a city. After about twenty minutes of confusion the vanguard began to advance slowly into the plowed ground, and in just two hours they had reached the other side. During all this time about one hundred and fifty ants which had started from the aphid tree to the nest were collected near the border of the plowed ground, but not one of them ventured as much as an inch into it. The next day and evening they were traveling back and forth just as if nothing had happened, and the path that they followed was so straight that when I stretched a line across between the two points of entrance no ant was seen to be more than five inches to either side of the line anywhere along the course. A decided curve was made in the path, however, after reaching smooth ground on the side toward the aphid tree.

Experiment I.

December 24, 1906, I connected a Fielde nest containing a colony of *C. pennsylvanicus* with a feeding-room by means of a series of five glass tubes, each about six inches long. Five days later, when the ants had become accustomed to these tubes, I turned one of the sections of the tube end for end and ants passed through in both directions without seeming to notice the change. Next I removed one of the sections and replaced it with a new piece of tubing of the same size. At this time three workers were in the feeding-room, and soon one of them started to go to the nest. She went hurrying along the tube until she came to the new section, when she suddenly stopped and began feeling cautiously about. She then made several trips to the feeding-room and back to the new section, but did not venture a full length into it. While she was continuing in this way another worker came from the nest and she too came to a sudden stop on reaching the new section of the tube. She examined it carefully and then, without returning to the nest, proceeded cautiously through it. Here she met her friend who had formerly discovered the change, and after they had exchanged antennal greetings, the two returned to the nest. This experiment was repeated a number of times and always with the same results, that is, those ants which were in the feeding-room always refused to cross the new section until they had met some friends directly from the nest, while those coming directly from the nest always crossed the new section, at least, after making one trip back to the nest.

Experiment II.

The same nest as above was connected with the feeding-room by means of a glass tube four feet long which had been bent at the center to form an angle of about 110°. The ends were bent in the opposite direction, so that by slipping the ends of this tube over the smaller tubes which led from the nest and feeding-room respectively, and by rotating the larger tube about the smaller ones as axes, I could vary the elevation over which the ants must travel in passing from nest to feeding-room. The tube was allowed to lie flat on the table usually, and at intervals I would raise it up so as to cause a steep incline. When this was done

ants which were in the feeding-room would not hesitate to through it, but they would invariably try to walk on the which was lowermost when in the usual position.

Experiment III.

I have repeatedly taken a stick and made a narrow mar the earth across the path of the ants from the nest to the a tree and have observed that only very few of the ants going f the nest to the aphid tree seem to notice it, while nearly all t going in the opposite direction would stop and examine the n carefully for some time, and some would return to the aphid rather than cross the mark.

These experiments show clearly that the ants behave differe when traveling from the nest than when returning to it. T are seemingly willing to venture into new territory when tra ing away from the nest, because of something akin to a sciousness that they can at any time retrace their steps and the nest, while when traveling toward the nest the link is br when the surface of the earth is disturbed across their p This may be either because the continuity of the outgoing tra destroyed, or because the appearance of things with which are familiar is altered.

Observation 2 seems to indicate quite strongly that these possess a sense of direction and an unusual power of usin under unfavorable circumstances. The rough ground all ma with weeds must have appeared to them much as a mour region over which a cyclone had torn the forest to shreds w appear to us, and yet they made a straight path across it in darkness. Their determination to cross this hazardous regi once seems also to imply that had some realization of the in ests at stake and some memory of the direction in which goal lay.

Experiment IV.

A Fielde nest containing a large colony of *C. pennsylva* was connected with a feeding-room by means of a system of glass tubes, each one half inch in diameter. One of these t was straight, another was bent into a vertical loop, another a horizontal loop, and the fourth was arched so as to form a s

incline. All four were brought together at the ends into triangular-shaped vestibules. A single tube led from one of these to the nest, and one from the other to the feeding-room. After this apparatus had been set up for forty-eight hours and the ants had become somewhat accustomed to the system of tubes, I placed in the feeding-room about two hundred larvæ which had been taken from the nest two hours before. Two small workers were in the tubes when the larvæ were placed in the feeding-room. They were apparently lost, for they divided their time between remaining perfectly motionless as if trying to gain their bearings, cleaning their antennæ, and running frantically about in the tubes. After about twenty minutes of such conduct, one of them entered the feeding-room and discovered the larvæ. She examined them carefully with her antennæ and then, with more excitement than before, renewed her search for the nest. She ran wildly about the system of tubes and the feeding-room for twenty-two minutes, and then found her way from the vestibule to the nest. On reaching the nest she ran against five of her companions very much as ants do when they first discover a stranger in the nest, and she then returned directly to the larvæ, passing through the straight tube. The five friends which had been greeted in this peculiar way turned around a time or two and then followed their informant immediately into the tubes, all passing into the straight tube, and three of them going directly to the larvæ in the feeding-room. The other two seemed to lose the trail in the second vestibule, and began running about the tubes. Each of the four who reached the larvæ began carrying them into the straight tube, and after making three trips from the larvæ to this tube, the original discoverer of the larvæ returned to the nest and, by the same behavior as before, succeeded in bringing three others to the scene of activity. Before all the larvæ were removed from the feeding-room five ants had returned to the nest for help and each time secured it. Thirty ants were in this way called into service, yet not an ant left the nest which had not been greeted in this peculiar way. After all the larvæ had been carried into the straight tube, the ants began carrying them into the nest, and as the larvæ arrived in the nest other ants joined in the work, so that the tubes were soon alive with ants.

This experiment was repeated a number of times and ea gave unmistakable evidence that the ant which discover larvæ in some way conveyed the intelligence to others. also had similar evidence of communication when I plac carcass of a white grub in the feeding-room of a colony th been deprived of animal food for some time. In the succ trials of this experiment, however, it never happened agai all the larvæ were returned to the nest through the straigh They were as often carried over the vertical loop as throu other tube, and this happened just as frequently after the ar used the tubes for three or four months as at first. Th always work as if in great haste to get the larvæ back to th and it seems that if they had had any discretion whatever would have chosen the shorter and less difficult route. instance, however, I watched an ant while making six trip a cluster of larvæ which had been carried into the straigh Three of the six times, when returning to the larvæ, she e the arched tube from the vestibule and proceeded until sh to the incline and then each time turned about and fou straight tube and the larvæ. She seemed to remember th larvæ were in the straight tube and so knew that she was wrong tube when she came to the incline. She also seem to be tracking herself as she returned to the larvæ.

Experiment V.

Three islands were formed by inverting two-inch Petri in four-inch ones and filling the larger ones with water. these islands, which I will designate as *A*, was connected wi feed-room of the apparatus used in experiment IV., by me a glass tube which was bent in such a way as to be part mersed in water in a Petri dish. This made it impossible f ants to crawl back to the feeding-room over the tube and to the table. The other islands, which I will designate as *C* respectively, were connected with island *A* by means of b of cardboard eight inches long and one half inch wide. placed in a direct line with the tube leading to *A*, and placed at right angles to the tube, opposite *A*.

Larvæ taken from the colony in the nest were placed on

above are typical. The principal variations are the following: Sometimes instead of waiting for an ant to find the larvæ, I took one directly from the nest and put her with them. When this was done the ant invariably picked up a cluster of larvæ and sought to find a temporary place of safety for them. In this she sometimes succeeded, and at other times she brought the larvæ back to the others on the island and then found the way home before again touching them. In no instance, however, did an ant which had found the larvæ herself attempt to carry them away until she had made a trip to the nest.

In one trial the ant which found the larvæ called on eight of her friends to help before she started to lead them to the larvæ. While she was doing this two of the first she had saluted started for the larvæ ahead of her and went directly to them, following exactly the route over which their informant had returned to the nest.

If I changed the bridge from *B* to *C* and replaced it with a new one while the first discoverer of the larvæ was on her first trip to the nest, those that followed her back invariably went to *C* and, not finding the larvæ there, they often returned to the nest and seemed to give up the search as if they had been falsely advised. But, although the original discoverer sometimes also followed her old trail to *C*, I never knew one which had really seen the larvæ to give up searching until she found them again.

I think that these experiments and observations fully warrant the following conclusions:

1. These ants have some means of inter-communication. A. Bethe has endeavored to show that all so-called communication among ants may be explained by odors carried by the informants and perceived by those saluted (Dürfen wir den Ameisen und Bienen Psychische Qualitaten zurchreiben). But in this case I do not see how the ants saluted could have known that the odor of the larvæ which the informant may have borne was not received from the larvæ in the nest. It is perhaps possible that the larvæ removed from the nest give off some special odor which is a signal of distress and which may be conveyed to the nest by the informant, but I think this far less probable than the other explanation.

2. These ants are capable of tracking themselves and others of the colony, but they are not capable of distinguishing the direction in which the trail was first laid down.

3. They do not depend entirely on following trails in finding their way, but are guided often by a kind of memory of the location of things, and probably depend as a last resort on a sense of direction.

4. They ordinarily pay very little attention to trails when traveling from the nest.

5. They give no evidence, in these operations, of anything akin to reason.

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BIOLOGICAL BULLETIN

A STATISTICAL STUDY OF MITOSIS AND AMITOSIS IN THE ENTODERM OF FASCIOLARIA TULIPA, VAR. DISTANS.[1]

O. C. GLASER.

INTRODUCTION.

Remak's ('41) diagrammatic schema of nuclear and cell division was banished from the field of normal biology by the cytological work of the decade following its proposal. Since that time it has ever remained heresy to associate amitosis of any sort with anything else than cellular senescence, or a high grade of specialization, or intense metabolic activity. "When once a cell has undergone amitotic division it has received its death warrant," wrote vom Rath ('91), and although this assertion is now acknowledged to be extreme, its spirit is nevertheless still so firmly engrafted on biological literature and thought that the uncanonical facts claimed by Pfeffer ('99) to obtain under experimental conditions in *Spirogyra*, and by Meves ('91) under natural conditions in the testis of the salamander have been regarded more as anomalies than as contributions to our knowledge of cell division. Quite recently however Child ('04; '07 I., II., III., IV., V., VI.; '07*a*) as the result of his very careful work on the cestode *Moniezia*, and his more or less exploratory observations on representatives of almost every phylum in the animal kingdom, has forced upon cytologists so many instances in which amitosis seems to occur in normal and healthy tissues, that the significance of what he found demands serious consideration. Appeal to inadequate technical methods, to senescence, to specialization, or to pathology are insufficient. Wheeler ('89) and

[1] Contributions from the Zoölogical Laboratory, University of Michigan, No. 114.

Osborn ('04, I. and II.), have also published data that have helped to reopen the old wound. It is again debatable what part amitosis plays in normal cell differentiation, and also whether a direct nuclear division may intervene between mitotic divisions without wrecking the ability of the cell in which it occurs to have progeny capable of further differentiation. In the present paper I intend to discuss the first of these questions on the basis of determinations quantitatively as exact as the nature of the subject and material permit. The technical methods employed in fixation, staining, and sectioning, have been fully described in an earlier paper (Glaser, '05). There also will be found evidence of the adequacy of the methods used.

Developmental Stages Considered.

The developmental stages which I have considered for the purposes of this work are those of the cannibal and veliger periods. The highly interesting events of this portion of the life history of *Fasciolaria* have been described in detail (Glaser, '05) but in order to facilitate the description of both the development of the entoderm and of the nuclear phenomena exhibited by this tissue, it will be necessary to restate briefly the chief facts in the gross embryology.

The entire development of *Fasciolaria* is influenced and modified, either directly or indirectly, by the process of cannibalism. This form of embryonic nutrition seems to depend on three things: on the fact that the eggs are laid inside of capsules; that thousands of them remain unfertilized; and that the embryos within each egg-case differ markedly in age, in size, and in vigor. Given these circumstances, the most vigorous larvæ within each capsule ingest all of the infertile eggs and all of the weaklings. Stages intended to illustrate typical degrees of cannibalism are shown in the second column of Fig. 9, p. 233.

Larva I. is the earliest stage used. It shows the mouth between the two bulging external kidneys, and contains under the right one, remnants of the macromeres of the segmentation period. Farther down in the digestive tract lie two of the swallowed food-ova.

Larva II. has ingested fourteen eggs, whereas III. is a fully

gorged and distended cannibal. The lower two larvæ, IV. and V., represent stages is the development of the veliger. I have not attempted to show the ova with which they are filled, nor is it necessary at this time to discuss the external changes involved in the transformation of a cannibal into a veliger.

The Development of the Entoderm.

It will prove to be an advantage if the description of the development of the entoderm is begun at a stage earlier than I., Fig. 9. A transverse section through the earliest larva available for the present purpose is shown in Fig. 1. The section is bilaterally symmetrical and shows on the right and left, the beginnings of the external kidneys (*ex.k.*). Beneath these rudiments, is mesoderm (*mes.*) with indistinct cell boundaries, while under this layer and immediately upon the yolk, is the entoderm (*ent.*), as yet an incomplete membrane composed of a few spindle-shaped cells with extremely attenuated processes.

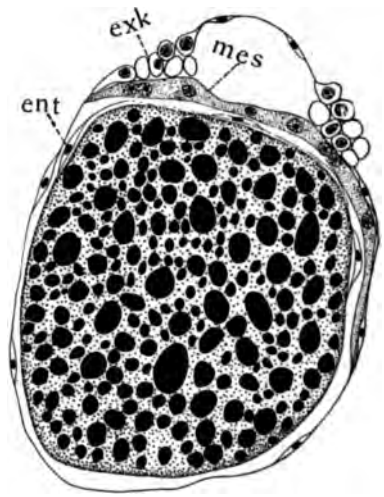


Fig. 1. A transverse section through a young pre-cannibal, showing the external kidneys (*ex.k.*); beneath these the mesoderm (*mes.*); and immediately upon the yolk, the spindle-shaped entoderm cells (*ent.*).

Fig. 2, a section cut in plane *xy* of stage I., Fig. 9, illustrates the cellular conditions met with at the beginning of cannibalism. Cell boundaries in all of the tissues except the external kidneys (*ex.k.*) are obscure. The ectoderm elsewhere is a spongy syncitium, varying considerably in consistency in different regions. The entoderm is apparently also a syncitium, but is spongy only in the anterior region *A* where it is impossible to define its limits. Ventrally *V* on the side toward the external kidney, posteriorly *P* diammetrically opposite the cap of spongy ectoderm, and dorsally *D* diammetrically opposite the external kidney, the

entoderm exhibits granulated nuclei imbedded in a gr
sometimes slightly alveolar ground substance in whic
boundaries are indistinguishable. All the nuclei are surrc
by a zone in which the particles are exceedingly dense, b

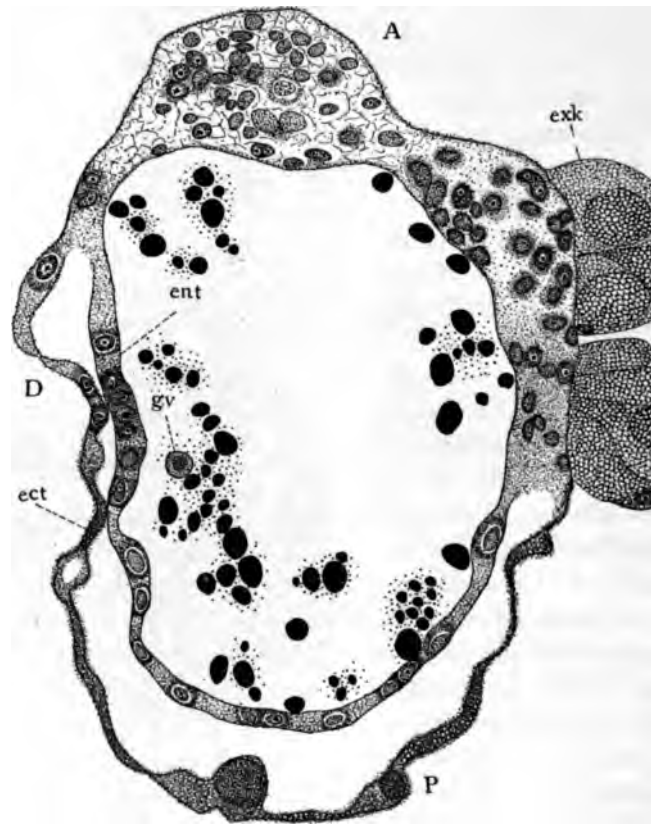


FIG. 2. A longitudinal section cut in plane *xy* of stage I., Fig. 9. On
(ventral, *V.*) is shown the external kidney (*ex.k.*). Anteriorly *A*, w
ectoderm (*ect.*) and the entoderm (*ent.*) meet is the cap of spongy tissue desc
p. 221. *G.v.* is the fragment of a germinal vesicle from one of the food ov
the difference between the entoderm in this stage and that characteristic of tl
and later larvæ.

region does not always abut upon the nuclear membrane
many cases therefore a narrow clear band devoid of granu
be seen between the nucleus and the dense zone. C
nucleus is found to contain a nucleolus, at times surrounc

Here too Osborn found hiatuses, but if these really occurred in the living state, it is difficult to see how a sac with holes in both its inner and outer linings could contain the eggs which these larvæ ingest.

When the fully gorged cannibals transform into veligers, the changes undergone by the entoderm are as striking as those in the external form of the larvæ. These changes lead to regional differentiation, the outcome of which is that the dorsal cells of the digestive tract come to be very unlike the ventral ones, whereas between these two zones, laterally, there are transitional cell forms. In addition to this morphological differentiation which holds true of the digestive tract from its most anterior end back to the region where it becomes identical with the digestive gland or liver, there is a well-marked physiological differentiation between the cells in the œsophageal region and those posterior to this zone. Fig. 4 shows a section, based on the study of several, through the œsophagus. The lumen of the tube is lined by comparatively small cells, provided either with several nuclei, or with lobed ones. The cytoplasmic contents of these cells are quite granular, and are often so densely crowded along the inner surfaces of the cell membranes that the nuclei in these cases seem to float in clear lakes of non-tingible cell sap.

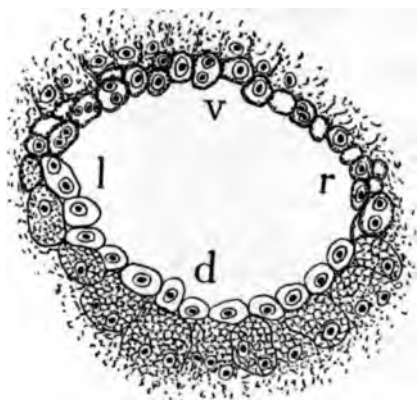


FIG. 4. A transverse section through the œsophageal entoderm of a larva in stage IV., based on the study of several sections through this region. *L*, left; *r*, right; *v*, ventral; *d*, dorsal.

The outer border of the œsophagus has a very different appearance. The cells there in many cases show unmistakable signs of disintegration, especially ventrally *v*, where often cell-fragments and quite isolated nuclei can be seen. Dorsally *d* the outermost cells are very large, polynuclear, frequently without complete cell-membranes, and their contents which are granular,

occurs in all healthy larvæ, and is only a part of a normal, but highly peculiar developmental history.

Well posterior to the œsophagus, transverse sections also exhibit two very distinct kinds of entodermal elements, although one finds intermediate stages between them. Ventrally *v* and partly laterally *l* the entoderm as compared with the dorsal cells is a thin layer; the cells are granular and vacuolated especially laterally, and except where there are transition stages into the dorsal cells, definite boundaries are not always recognizable. The striking condition of the dorsally situated liver cells is connected with digestion since they seem to serve as temporary storage places for digested or partly digested yolk. These cells are unusually large, and very remarkable in appearance. Their contents differ greatly in arrangement, and at first sight in their reactions with orange *G*, but such differences as they present in this respect are due to the density of the materials, and not to any fundamental difference in their composition. Certain irregular masses containing one or more large open spaces and many very minute ones, tinge deeply and are frequently separated by an area of considerable width from what I take to be cell boundaries. These boundaries where clearly observable are made up of exceedingly fine fibrils closely packed. Among the other cell contents seen in this region are granules of two sizes, very minute ones not always regularly distributed, and somewhat coarser ones arranged in a reticulate manner. Both of these kinds of material stain with orange *G*, though on the whole less deeply than the dense masses with the large vacant spaces. In the lumen of the digestive cavity are granules of exactly the same staining reactions as those inside of the cells and these also are arranged partly without regularity, partly in reticula. Here and there are small collections of larger granules that suggest from their grouping fragmented yolk spherules. Since all of these materials, intra-, as well as the extra-cellular have the some staining reactions with orange *G*, I conclude that they represent stages in the digestion of yolk.

Laterally *l* and ventrally *v* the entoderm cells have a fundamentally different appearance from the liver cells; they are less definite on the whole in their outlines; are decidedly smaller in

size; contain no granules that stain with orange *G* and are occasionally almost completely filled with a vacuole, so that in certain localities I feel reasonably certain that two adjoining vacuoles often represent two cells. The nuclei of the entoderm in this region are small in comparison with those from other places.

The Nuclear Phenomena in the Entoderm.

The fact that amitosis occurs in the entoderm of *Fasciolaria* embryos, was so far as I know first definitely asserted by Osborn. "The entoderm," says Osborn ('04 I.), "is composed of cubical cells in which one finds all stages of direct division."[1] Fig. 6 represents some of these divisions. The nuclei shown in this picture were enlarged from the same sections from which Fig. 4 was compounded. *A* and *b* are removed from their cells. In one of them *a* the finely divided chromatin granules exhibit a slightly reticular arrangement and considerable condensation along the inner surface of the nuclear membrane. Here and there are larger dense collections of these granules suggesting an interrupted skein. The nucleus in question is markedly bilobed, the larger lobe having a small nucleolus, the smaller lobe a large nucleolus. Separating the two lobes incompletely is a very delicate interrupted membrane, which on close inspection was found to be composed of a dense collection of granules like those lining the inside of the nuclear membrane. I have seen these granular boundaries so frequently between the lobes of what I take to be dividing nuclei, that I conclude that cleavage is in many cases initiated by a granular plate that grows inward from the nuclear wall. Nucleus *b* is very much

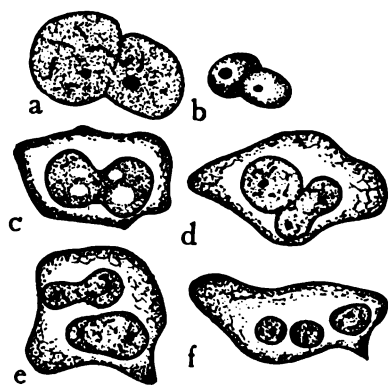


FIG. 6. Cells and nuclei from the excretory zone of the entoderm.

[1] These direct divisions were interpreted by Osborn in a later paper ('04, II.) as growth phenomena, a view supportable, as the sequel will show, by much additional evidence.

smaller than *a*, is also bilobed, and the lobes contain different sized nucleoli. *B* appears to differ from *a* in three striking details: the finely divided chromatin is not arranged in a reticulum; there are no larger chromatin bodies and the nucleoli are surrounded by large clear areas devoid of tingible material. The remaining nuclei and their cells (*c*, *d*, *e*, *f*), illustrate the conditions most commonly met with in the disintegrating cells. The cell contents, irregular masses of granules and what appear to be fibrils or strands, are crowded along the inner surfaces of the cell membranes and are separated by clear regions from the bilobed or dividing nuclei that occupy approximately the centers of the cells. These nuclei differ markedly in several respects from those already described. Their granular contents are not clearly reticulate; such large masses of chromatin as they contain are much condensed and the nucleoli often have definite chromatin radiations, a condition suggesting that all of these nucleoli are chromatin nucleoli, especially as *b* shows no other large chromatin bodies. In addition large vacuoles are often found inside of the nuclei.

The direct divisions to which I have devoted most of my attention occur in those regions of the entoderm where neither liver nor disintegrating cells are found. The nuclei there (Fig. 7) are not remarkable for size, in fact they are rather small, a condition which favors the view that they are not very active metabolically. They may or may not exhibit nucleoli, and these may or may not be surrounded by halos devoid of chromatic material. The nucleoli are usually small and their staining reaction is different from that of the other nuclear contents. The chromatin is usually scattered irregularly in the form of granules somewhat larger than those of the other amitotic entodermal nuclei. Some of the nuclei show clear spaces independent of the nucleoli, but these regions of achromatic material are not always sufficiently distinct to warrant the same interpretation for all. Some seem to be vacuolar; others are certainly not. Many of the nuclei contain two nucleoli. These may differ in size, and may lie rather close together or be separated by a considerable distance. I have never seen such nucleoli in the act of division. Among these nuclei I have found what I interpret as all possible stages

of amitosis, and the nineteen represented in Fig. 7 are cases some of which one can find in every section.

I have not been able to convince myself that there is any particular way in which these nuclei divide, on the contrary, the details of their division vary considerably and there may be others of which as yet I have no inkling. Figures such as 2, 5,

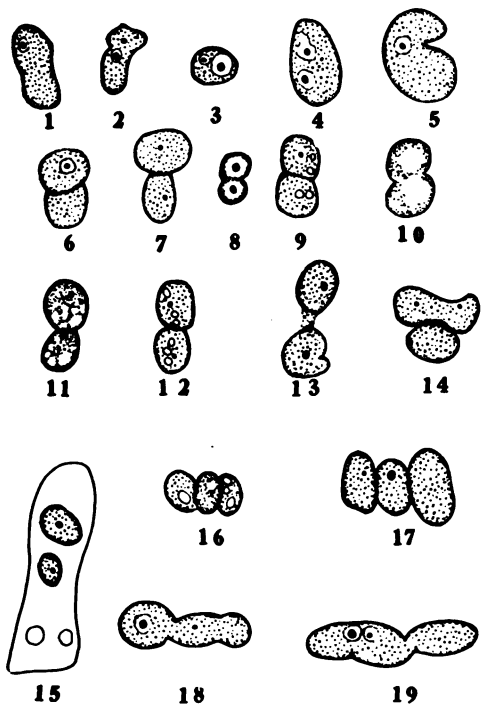


FIG. 7. Nuclei from the ventral and lateral comparatively undifferentiated entoderm in the digestive zone of stage IV. and later stages.

10, 13, suggest that the process of division may begin by the formation of a lobe, and that this lobe may then be gradually constricted off. The nuclei that one finds close together, such as 16 and 17 often differ greatly in size suggesting that the lobes from which they came may have been unequal, a condition actually observed in many instances. Number 13 is a most interesting and valuable nucleus, because it shows beyond doubt a slightly chromatic, somewhat attenuated bridge connecting

two widely separated lobes, one of which — the lower — has the nuclear membrane equally distinct throughout its circumference. This nucleus was killed in the very act of pulling apart. Other nuclei such as 6, 7, 8, 9, 11 and 12, seem to be dividing by the formation of a granular plate, such as is exhibited by some of the nuclei in Fig. 4. Others, such as 14 and 15, the latter drawn with its clearly marked cell boundaries, give no indication whatever of how the separation may have taken place. The groups 16, 17, 18 and 19, are extremely interesting as they seem to throw light on the origin of nuclear nests. Very frequently I have found three, four or five nuclei huddled together so closely that I could make out clearly no other relation between them. Often one of them is at a slightly different level from the others. In the cases under consideration the history of such nests may be read. A nucleus instead of dividing into two, in the manner of an amœba, simply elongates, and becomes lobed in two or more widely separated regions which may or may not be provided with nucleoli. These lobes later separate, and the original nucleus has divided into three or more parts, approximately equal in size or at times quite unequal. That there is nothing anomalous about this mode of division is illustrated by the nuclei in the external kidneys in which one frequently finds these conditions clearly exemplified (Fig. 8). In comparing the nuclei

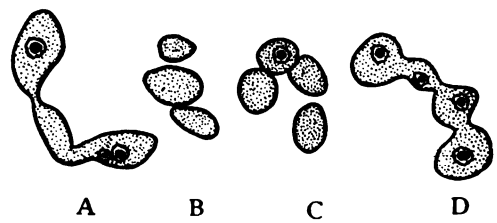


FIG. 8. Nuclei from the external kidneys where cases of multiple simultaneous division are frequent. These drawings were made from entire nuclei and show that the divisions are not dependent on the activities of the nucleoli, which may or may not be present.

just described with those in the disintegrating entoderm cells, it is clear that, excluding 17, 18 and 19, they are very much smaller in size. Furthermore, the nuclei in Fig. 7 show none of the chromatin masses exhibited by the nuclei in the disintegrating

cells, and there is no morphological indication that the nucleoli contain chromatin, as they never exhibit the radiations found so frequently in the former group. As a consequence probably of the absence of chromatin nucleoli and the other larger chromatic masses seen in the nuclei of Fig. 6, such granules as one does find, are slightly larger than the finely distributed chromatin of the nuclei in the disintegrating cells. The two kinds of nuclei therefore exhibit certain well-marked histological differences, and these differences make it comparatively easy not to mistake the one kind for the other — even in the transitional regions where both occur together — regions which I eliminated altogether from the determinations.

Difficulties.

The interpretation of the histological facts given in the preceding section offers difficulties some of which inhere in the material used, while others inhere in the subject, and would be met with no matter what animal was studied. In the first place, the technical difficulties encountered in attempting to cut serial sections were such that my series are only rarely complete and hence unsuitable for the determination of the total number of nuclei per embryo. I was able however to determine the total number of nuclei in each section, and to count the resting ones and those dividing either directly or indirectly. Each section was thus treated as an independent entity without regard to what preceded or followed it. The results therefore show that in the particular set of sections which I studied, each one treated individually, a certain number of nuclei were dividing directly and a certain number indirectly. The relative frequencies of mitosis and amitosis are in no wise altered by the imperfections alluded to.

The second difficulty that was encountered, was the physiological differentiation of the entoderm into an anterior excretory zone and a much larger posterior assimilative zone. While complicating the problem to some extent, the regulative disintegration brought on by intense excretory activity, is restricted to a very definite region, back of which nothing like it was ever observed. It is necessary of course to conclude that some of the entoderm cells are temporary larval structures, but this conclusion should not be extended so as to include the entire entoderm.

If the lining of the entire embryonic digestive tract were temporary, one should be able to find reserve elements from which at a later stage the definitive entoderm might be derived. Careful search has failed to reveal such cells. Even granting that such reserve cells do indeed exist, but that they are not sufficiently well characterized to attract attention, there are no regions in the entoderm in which amitosis is absent, and the assumption that there are reserve cells involves of necessity the belief that the definitive entoderm comes from cells like those described and figured. Since there are constant histological differences between the nuclei in the two regions under discussion, and further since the disintegrating cells are very definitely restricted, they can be eliminated from the field of inquiry by tracing them to their posterior limits and considering only cells well back of this boundary.

A third difficulty was encountered when it was found that not only is it impossible to cut mitotic figures and amitotic nuclei serially into an equal number of sections, but they cannot even be sectioned in an equal variety of planes that will reveal their true character. Actual measurements, as well as experiments with models representing direct and indirect nuclear division, show that when nuclei are equal in volume, one in anaphase can be cut in many more planes that will reveal its true mitotic character, than an amitotic nucleus of equal mass. In fact in very late stages of amitosis, stages in which the daughter nuclei are connected with one another by very small or very attenuated bridges, only planes passing through the long axis of the dumbbell shape will exhibit the true relation of the lobes. Since the amitoses probably take place in all possible planes, the error due to the above factors is no doubt a considerable one.

A fourth difficulty needs to be considered, namely, the possibility that the larvæ studied were abnormal. To eliminate errors due to this source I used more than one embryo in each of the stages represented in Fig. 9, except the first two, of which no greater number was available. Since the argument, as the sequel will show, does not hinge on individuals, but on a comparison of the first half of the developmental period considered, with the second half, the scarcity of early stages is compensated for. Thus

the results are actually based on three larvæ of the cannibal period, and on four of the post-cannibal period though many others were used for comparison.

A final difficulty not at all peculiar to *Fasciolaria*, but to be expected wherever amitosis occurs, is this: How can one tell that what seems to be an amitotic division is really such? Since in amitosis there occur none of the striking changes that characterize mitosis, it is, as Hertwig ('98) has pointed out, impossible to be sure that direct divisions are going on unless one can find all possible stages in the process. The mere lobulation of nuclei is not sufficient. I believe that Fig. 7 is an answer to the criticism which neglect of Hertwig's warning might justify. Of course, many of the nuclei there pictured would not have been included in the same plate with those which I cannot doubt are amitotic, had I not found the latter. Given stages however which it is impossible to interpret in any other way, it seems mere pedantry to exclude all of the others which taken by themselves, would either not be convincing, or to the casual observer, might not even suggest amitosis. Had it been impossible for instance to find all of the intermediate stages between a resting nucleus and a late metaphase, I doubt very much whether anyone totally ignorant of the process of mitosis would be able to assert that the latter stage had been derived from the former. The initial and final conditions however are safely interpreted in terms of the intermediate stages that have been found, and every step in the process is illuminated by every other step. However, I have chosen to err on the safe side, and while Fig. 7 includes all of the different nuclear forms met with, in the actual counts only nuclei like 6, 7, 8, 9, 10, 11, 12 and 13, were included. None of the nests, such as 16 and 17, were counted, nor the elongated forms, like 18 and 19, from which the nests may have been derived. Even nuclei as close together as 14 were not included, nor such as 15 in which the cell boundary enclosing them could, as is sometimes the case, be distinctly traced.

Summing up the effects which all of these difficulties and their evasion have on the final result, I think it may be justly said that the incompleteness of many of the sections is without significance; that the complete elimination of the temporary cells

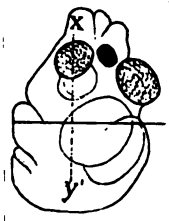
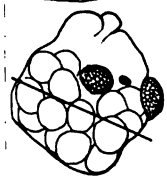
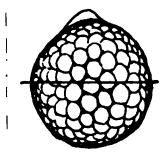
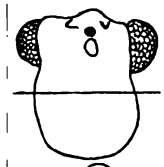
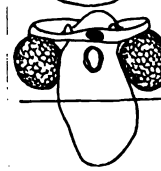
Stage.	Condition.	Sections.	Entoderm.	Nuclei.	Mitoses.	Amitoses.	Ce
I.		38	Cuboidal *a*	339.	17	21	2
II.		12	"	143	0	0	2
III.		65	Elongated	916	2	42	2
IV.		15	Cuboidal	589	1	28	1
V.		51	"	1,162	0	41	1
Totals				3,149	20	132	

FIG. 9. The first column at the left contains the numbers applied for purpos description to the several stages used; the second column contains outline dra illustrating the condition of the stages employed; the third the number of sec studied; the fourth a statement of the condition of the entoderm; the fifth the ber of nuclei counted; the sixth the number of mitoses seen; the seventh the nu of amitotic divisions registered; and the eighth the number of entodermal cells pr in a section cut in the plane of the heavy line which is drawn through each pi of the larval stages in the second column. Wherever number or other statement based on inference or deduction, this fact is indicated by a small letter which r to a foot-note, which in turn refers, if necessary, to the page where the eviden which the inference or deduction is based, is given in full. (*a*) See Fig. 2 and 238. (*b*) Estimated; see Fig. 2 and page 238. (*c*) Estimated; see page 23

involves also the elimination of a considerable number of permanent ones; and that the fact that mitoses can be cut in more planes, and also into a greater number of sections in any plane, than amitoses, and still reveal their true nature, increases greatly the percentage of indirect divisions in the determinations of the relative frequencies of these two forms of division. The fact that amitoses are much harder to recognize than mitoses, and that I counted as direct divisions only those that seemed to me unquestionable cases, also helps to increase the relative frequency of mitosis in the final determinations. It follows therefore that the methods employed give a maximum of mitoses and a minimum of the process that I interpret as amitosis.

The Relative Frequency of Mitosis and Amitosis.

The main results of my work are graphically illustrated by Fig. 9. There are arranged in tabular form, outline drawings of larvæ in the stages of development used, and on a line with each one are the number of sections on which the determinations are based; a statement concerning the condition of the entoderm; the number of nuclei actually counted; the number of mitotic divisions found; the number of amitoses registered; and the number of cells either actually found in the transverse planes indicated on the drawings, or inferred to be present there from evidence which will be given in detail for each case in which inference replaces actual counting.

Analysis of this table reveals several interesting facts. In stage I. for example, there is a higher percentage of mitoses, and of course a lower percentage of resting nuclei than in any of the other stages. Three years ago when I had worked out the relations between direct and indirect nuclear division in the entoderm, I had come to the conclusion that in the early stages the divisions in this tissue were predominantly mitotic whereas in the later stages the reverse was true. My notes in some way were lost but on repetition the same result, as the table shows, appeared. I thought at that time that the numerous amitoses in the later stages were connected entirely with digestion, and were of no significance in the formation of the definitive entoderm. Although not published in any journal I expressed this view in a

paper read before the Research Seminar at Wood's Hole in the summer of 1905. This view may still appeal to some as valid, but I think that certain facts then unknown to me point strongly in the opposite direction. While it is true that during the early stages of cannibalism the increase in the size of the embryos is mere stretching due to the ingestion of eggs, such stretching is not all that happens in the larvæ. It will be recalled that the entoderm in stage I. is a pretty thick layer in which cell boundaries are obscure. An examination of the layer however will show that if cell boundaries were distinct, the cells would be cuboidal, or rectangular or at most diamond-shaped (see Fig. 2). After the larvæ have taken on the form of stage III. the entoderm, as Fig. 3 shows, has an entirely different appearance, and the distinct outlines of the cells show that these instead of having the shape of cubes or rectangles, or diamonds, are spindle-shaped, and provided with long processes that by fusion with corresponding elongations from neighboring cells form a continuous membrane. I think that no special argument is necessary to support the view that the transformation undergone by the entoderm cells when the larvæ pass from stage I. to stage III., is the direct result of stretching. Such an effect is to be expected. This condition however is not final. The elongated entoderm cells soon lose the characteristics which they exhibit in stage III. and return to a condition more nearly like that in stage I., except for two general differences: boundaries are more distinct than in the younger entoderm, and regional differentiation is observable.

These metamorphoses are of great importance. If the larvæ decreased markedly in size during the later stages of their development, and as the result of such shrinkage approached the size of the pre-cannibals, the conversion of the spindle-shaped cells of stage III. into the cuboidal cells of stages IV. and V. could be attributed to this cause: to decreased stretching. The idea that the later stages in development might be smaller than the earlier, is somewhat bizarre when viewed in the light of our knowledge of ontogeny in general, for the exact reverse is law. Nevertheless such decrease in *Fasciolaria* embryos is quite easily conceivable as the size depends on what the larvæ contain. If metab-

olism were very intense, and the swallowed yolk were very quickly used up, instead of remaining in the digestive tract for five or six weeks as it actually does, the elimination of waste products might be rapid and great enough to bring about an actual decrease in the size of the later stages. Such shrinkage would indeed occur were the effects of the digestion of yolk and of the elimination of wastes not more than compensated for by four factors, three of which obtain in every individual, whereas the fourth is operative only in the majority of cases. In the first place, after the period of cannibalism, the larvæ actually increase in size by ordinary growth; in the second place, even after completely filled with eggs, they continue to inflate themselves with the albuminous material in which they float; and thirdly several days after ingestion many of the eggs lose their pellicles, and since the yolk granules are large, very firm, and vary considerably both in size and shape it is to be expected that they would take up more room than when neatly packed, as they are, in the intact ova. In addition to these factors of enlargement, one very remarkable one operates in so many cases, that it may be called a matter of common occurrence. In every capsule, practically, some of the cannibals in stage III. burst, and in those egg-cases in which only two or three larvæ in later stages are found, the majority of cannibals have broken. In these instances the surviving larvæ are invariably larger than those in the more populous capsules. Experiments have shown that a fully gorged cannibal, which under other conditions would have ingested no more eggs, will double the number it contains if the food supply is increased. From these experiments, as well as from the observation that where embryos are below the average in number they are above the average in size, and the further observation that bursting accidents occur in practically every capsule, it follows that after the stretching which transforms stage I. into stage III. has occurred, a further increase in size depending upon the factors mentioned takes place.

During the particular period of development now under consideration the activities of the entoderm are such that in spite of the stretching due to all of the causes mentioned, the cells of the inner layer change from the spindle-shape to the cuboidal. The

only way in which such a change can conceivably take place seems to me to be by a very rapid increase in the number or the size of the cells. An increase of the former sort can very readily be noticed during the period when the spindle-shaped cells of the early pre-cannibals (Fig. 1) change to the "cuboidal" cells of the young cannibals (Fig. 2). If a similar increase takes place when the fully gorged cannibals transform into veligers one should be able to find histological evidence of it. While the original transformation may be accounted for on the basis of 17 mitoses per 339 nuclei, the second transformation, if mitosis is the only method of division in normal cell differentiation, must be accounted for on the basis of 1 mitosis in 1,751 nuclei. This single case is absolutely the only indication of mitosis, that careful and frequently repeated search through stages IV. and V. has revealed. On the other hand I found in the same sections 111 cases of what I interpret as undoubted instances of amitosis. If the amitoses do not account for the increase of cells needed to explain the change from the spindle to the cuboidal shape, I doubt very much if the all but total absence of mitosis accounts for the facts. It is necessary to conclude therefore, either that the only form of division seen in any quantity is responsible for the assumed increase in cells, or that these enlarge and become comparatively crowded.

The crucial question then is, which of these two explanations is correct? Do the cells become crowded because they increase in size, or in number or for both reasons? The drawings show plainly that the dorsal cells do increase in size during the metamorphosis; they also show that this is not true of the ventral cells. In addition to the enlargement of the liver cells it can be shown that actual increase in the number of cells present, is a factor bringing about the change from spindle-shaped to cuboidal cells.

Absolutely faultless series, or strictly comparable sections are in several cases unavailable. Of stage I. for example, I have no transverse sections, but a study of the differences between the long axis and the short one of this larva makes 20 cells in a transverse section midway between the extremities of the longer axis, a safe estimate (see Fig. 2 and stage I., Fig. 9). In stage

II. actual counting of complete sections in an approximately comparable region, viz.: midway between the extremities of the long axis, gave 20 as the number of cells, whereas for stage III., 15 was the average of five incomplete sections taken near the plane of the equator. It is practically impossible to secure entire sections through larvæ in this condition on account of the thinness of both the body-wall and the wall of the digestive tract, neither of which is thicker, in many regions at least, than the pellicles around the ingested food-ova. In the sections from which the particular estimate now under consideration was made, one third of the circumference was incomplete; as there were fifteen cells in the other two thirds, I assumed that the torn region represented a distance which in the entire embryo was covered by five cells, an assumption justified by the study of other sections.

A comparison of stages II. and III. may suggest at first sight that the younger embryo should have fewer cells than the older one, since the latter contains so much more material than the former. Professor Osborn says that there are not enough cells in stage III. to enclose the food-ova. This however is a mistake; there are enough cells, only in order to "cover the ground" these are stretched almost beyond belief. Indeed the elongations are frequently as attenuated as the delicate projections so characteristic of mesenchyme cells.

The number of entoderm cells in stages IV. and V. was determined in complete sections, for the body-wall as well as the wall of the digestive tract have thickened so much in these older embryos that it is a comparatively easy matter to section them without injury. As the table shows, 57 cells is what I found in the younger of the two oldest stages and 93 cells in the oldest of all of those considered.

Granting for the sake of argument that the number of entoderm cells in the earlier stages is twice as great as my determinations indicate, the later stages would still show double the number of cells in corresponding regions. Fig. 2 shows that such an error is impossible. I am certain that the figures actually given are much nearer the truth and that instead of having twice the number of entoderm cells, the later stages of the de-

velopmental period considered have four times as many. As the period during which this increase chiefly occurs exhibited but one mitosis among 1,751 nuclei, the conclusion is practically forced on one that amitosis is the method of cell multiplication that obtains in the entoderm.

This conclusion however must be critically tested. Is there any possibility that after all one mitosis among 1,751 nuclei is enough to account for the facts of growth? This question can, I think, be definitely answered in the negative.

The time taken by a larva in stage III. to change into stage IV. is 13 days ('05). During this period, according to the determinations, the number of cells in the transverse planes under consideration increases from 20 to 93, an addition of 73 cells. Let us assume for the sake of argument that a complete mitosis, beginning with a resting mother nucleus and ending with two resting daughter nuclei can be accomplished in one minute. As $\frac{1}{2}\frac{1}{0}$ per cent. of the nuclei are dividing mitotically, it follows that in one minute .0005 mitosis occur. In 2,000 minutes therefore one complete mitosis would take place. Since 2,000 minutes equal 33 hours, it follows that once in this number of hours an entoderm cell would divide. Now the developmental period under examination endures 13 days, or 312 hours. If therefore one division takes place every 33 hours it follows that 9 such cleavages would occur in the 13 days. As the larva has "20" cells to begin with, the first division would raise this number to 21; the second to 22; and the ninth to 29. Thus if mitosis occurs at the determined rate of $\frac{1}{2}\frac{1}{0}$ per cent., and at the assumed speed, 9 new cells would have been produced. The actual counts show that 73 cells are added. Even if we double the speed and assume that a mitosis can be completed in 30 seconds there would still be a disparity of 55 cells. If this reasoning is correct, mitosis occurring with the frequency actually determined, is totally insufficient to account for the observed facts of growth.

One chance however remains. It is possible that my determinations of the frequency of mitosis during this developmental period are misleading; that I missed the epidemics of division, three of which would more than explain the facts, for if all of the 20 cells in stage III. were to divide at once the number would

be doubled; the second epidemic would yield 80 cells and the third 160. A less severe epidemic, one having more probability in fact, might exactly account for the approximately fourfold increase observed.

The assumption that epidemics of mitosis occur, but have been overlooked, is unfortunately without foundation. In the larvæ used for the determinations of the relative frequencies of mitosis and amitosis, as well as in the many others used as checks in working out the more strictly embryological details, I have never observed any indications of such epidemics. Similar indications seem also to have escaped Osborn.

It is impossible on the basis of such negative evidence as is available to assert dogmatically that they do not occur. My results however have some significance in this connection. A comparison of stages III., IV., and V., shows that I found 2 mitoses, 42 amitoses, and 20 cells in stage III.; 1 mitosis, 28 amitoses, and 57 cells in stage IV.; and 0 mitosis, 41 amitoses, and 93 cells in stage V. It might be asserted that the divisions which account for the increase from 20 to 57, took place during an epidemic of mitosis at some time between stages III. and IV. It might be claimed also, that the increase from 57 to 93, had come about as the result of a similar epidemic between stages IV. and V. These stages were selected because their external characteristics mark definite steps in the acquisition of the adult body form. Stage IV. is approximately a half-way station between stages III. and V. It is important therefore that in other respects also the larvæ should be half-way between the two extremes, for this is at least an indication of an even tenor in the rate of all of the developmental processes. Were growth spasmodic and not uniform, it would be very curious that the number of entoderm cells in a corresponding cross-section of the "half-way" larva should be 57, for the mean between 93 and 20 is 56.5.

The view that growth is uniform in rate gains in validity when we consider the percentage of indirect and direct divisions which occur during this crucial period. For stage III. the former is .2 per cent., the latter 4 per cent.; for stage IV., the former is .1 per cent., the latter, 4 per cent.; whereas, for stage V., we have 0

per cent. of mitosis, and 3 per cent. of amitoses. Allowing for errors, there is practically no fluctuation in the frequency of either mitotic or amitotic division in these three stages of development Since this is true, to say that the rate might have been very different between stages III. and IV., and again between stages IV and V., may be true, but is supportable by neither facts, nor probability. Indeed, it is not going too far to say that the percentages as well as the number of cells found, indicate the exact opposite, namely: that in this tissue, at this particular period of development, mitosis and amitosis occur at constant frequencies

Considering the table as a whole, it follows that of 3,340 nuclei, a little less than .6 per cent. exhibited mitotic figures If my interpretation of what constitutes amitosis is correct, there a little over 87 per cent. of all divisions are direct, whereas only a trifle more than 12 per cent. are mitotic. As I have pointed out before, these figures undoubtedly contain a large error due to the fact that early as well as late stages in amitosis are not sufficiently well marked to enable one to decide whether they belong into this category or into that of the resting nuclei. As all doubtful cases were relegated to the latter group, I feel confident that 87 per cent. represents the minimum of amitosis, and that in all probability the direct divisions are more frequent. In view of this I think that the conclusion is justified that amitosis is the chief mode in which the nuclei and cells increase in number. Of the two alternatives which these results allow, one, the possibility of epidemics of mitosis, is not only unfounded, but improbable; the other, namely, that a four-fold increase in cells can be accounted for on the basis of 1 mitosis in 1,751 nuclei, involves an absurdity.

Discussion.

I do not propose to enter at this time into an elaborate discussion of either the literature on amitosis, or of the theoretical questions on which direct nuclear divisions are thought to bear. The former has been very ably done by other writers, notably Henneguy ('96) and Wilson ('02), the latter I shall do after I have accumulated more data. The belief that in the entoderm of *Fasciolaria* we have an instance in which amitosis plays an important if not the chief part in the differentiation of a definitive

tissue, can however be supported by several references in the literature, and these I shall at least mention.

I have already referred to the work of Meves ('91) on the spermatogenesis of the salamander. In this well-known paper evidence is brought forward which shows that in the spermatogonia amitotic divisions take place during the fall, and that these succeeded in the following spring by the usual maturation phenomena, are part of the cycle of a normal organism. Wheeler ('89), in his paper on the embryology of *Blatta germanica* and *Doryphora decemlineata* has reached similar conclusions. Thus in *Blatta*, cells originate in the center of the ovum by mitosis. These cells are amœboid, and wander to the surface of the egg where they flatten out. "The cells which have reached the surface and are much scattered over the roof-shaped ventral face and the adjacent portions of the lateral faces commence dividing longitudinally, not by karyokinesis, as heretofore, but by akinesis." "My observations," continues Wheeler, "tend to show that all of the future divisions in the formation of the blastoderm, and those subsequently undergone by the serosa, are akinetic, the densely coiled chromatin filament remaining inert and the divisions taking place by a constriction which often produces two daughter nuclei of very unequal size. I emphasize the fact that these forms of division could not have been produced by the reagents, as the eggs were hardened in picro-sulphuric acid or simple alcohol, which in younger and older eggs preserve the karyokinetic figures of the cleavage nucleus and its immediate descendants with great clearness." From this it follows that the cells that make up the germ-layers from which the definitive cells of the body come, are all descended from cells which at an earlier period of development divided by amitosis.

Frenzel ('92) came to the conclusion that amitosis plays an important rôle in the regeneration of the intestinal elements in the crustaceans, and insects, for he claimed at first never to have found any indirect divisions. As Henneguy ('96) pointed out after Frenzel himself had corrected the mistake the conclusion that mitosis does not occur in the cells in question is undoubtedly incorrect, but the fact that the digestive tract in certain arthropods can be studied carefully without revealing any mitotic divisions,

shows at least that these must be rare. The same thing may be said of embryonic tissues in general, as Child has emphasized. Who has not been struck by the comparative scarcity of mitosis in tissues which are known to grow with great speed?

As implied in the introduction to this paper, whether amitosis plays a part in normal cell-differentiation, and whether direct divisions may intervene between indirect ones, without inhibiting further differentiation are really two distinct questions. In practice however it is impossible to keep them separate, for if amitosis does play a rôle, it does this in a normal tissue, and it is characteristic of normal tissues that their component cells at some time exhibit mitosis. The results both of Meves and of Wheeler offer cases in point. The same is true of Child's work. In *Moniezia* also cells which are part of an apparently normal cycle divide at one time amitotically (oögonial and spermatogonial divisions) and later mitotically (maturation divisions). Similarly after fertilization, the first cleavage of the egg is accompanied by a typical mitosis, whereas the later cleavages may be amitotic. Since the cells of the cleavage period are the ones from which the definitive structures of the adult come, it follows that amitosis plays a part in normal cell differentiation.

Neither *Moniezia*, Child's form, nor *Fasciolaria* are ideal animals to work upon, for aside from the mere matters of technique which in one of them offer considerable difficulty, both of these forms exhibit in the tissues studied (entoderm; ovary; testis) degenerating cells, and a certain number of mitotic divisions along with the amitotic ones. The possibility therefore exists that the indirect divisions are the really important ones, whereas the amitoses are physiological, and of no consequence in a genetic sense. According to Wheeler *Blatta* must be ideal for "all of the future divisions of the blastoderm and those subsequently undergone by the serosa are akinetic." Apparently here there is no chance of a mistake. In the absence of other forms equally well adapted for our purposes, there is only one thing to do — to measure as accurately as possible the frequency of the direct and indirect divisions in a tissue, and then on the basis of these measurements to see if the facts of growth that need explanation can be explained when one or the other of the two forms of division is ruled out.

This is what I have tried to do in the case of *Fasciolaria*, and what seems to me ought to be done in other forms. Merely stating that mitosis and amitosis occur, without also stating how frequently, does not meet the requirements of the problem.

If his interpretation of the life history of *Amœba proteus* is correct Calkins ('07) has advanced an absolutely conclusive case in which direct nuclear division is a link in a normal life-cycle. Calkins believes he has found evidence adequate to show that in *Amœba proteus* an asexual period is succeeded by a sexual one inaugurated by amitotic multiplication of the nucleus. The "primary nuclei" thus formed fragment and change to minute granular "secondary nuclei." The secondary nuclei later conjugate giving rise to the "fertilization nuclei"; "in these the fused karyosomes fragment to form finely divided chromatin (it is strictly speaking, not a chromidium for it is entirely intranuclear), while a vacuole forms in the interior; this vacuolated fertilization nucleus becomes a center of multiplication (equivalent in every way to a sporozoön sporoblast); by accumulation of these fine chromatin granules the peripheral or 'tertiary' nuclei are formed; the tertiary nuclei, surrounded by a minute bit of plasm, grow into the pseudopodiospores observed by Scheel (hypothetical); these young pseudopodiospores break away from the parent cyst and develop into young amœbæ fromerly known as *Amœba radiosa*, and these in turn develop into the ordinary *Amœba proteus* of pond and laboratory." If this represents truthfully the life cycle of *Amœba*, we have at least one conclusive case in which amitosis cannot be ruled out, for here there are no mitoses. Neither are there any degenerating cells to cast the shadow of suspicion on the other cells. Equally conclusive cases can hardly be hoped for among the higher animals, although what seems to be true for *Amœba*, may be also true of multicellular forms. If it proves impossible to establish these facts with mind-compelling certainty, further investigation should be able at least to endow them with a degree of probability amounting to a practical demonstration.

SUMMARY.

1. During the period of cannibalism, the entoderm of *Fasciolaria* becomes first spindle-shaped, but later as regional differentiation occurs, the cells become cuboidal.

2. The first change can be accounted for by the stretching which the larvæ undergo; the second change is explained by a fourfold increase in the number of cells found in transverse sections through the middle of the digestive tract.

3. During this period of cell increase there was found a maximum of one mitotic division in 1,751 nuclei.

4. During the same period of development, there was found a minimum of 69 amitotic divisions.

5. From this it follows that during the period of most active cell multiplication more than 1 per cent. of all divisions is mitotic and more than 98 per cent. are amitotic.

6. Since there were found during the pre-cannibal, the cannibal, and the post-cannibal periods, 152 cases of what is interpreted as nuclear division, and since of these 20 were mitotic, it follows that during the entire developmental period considered a little over 13 per cent. of all the divisions were mitotic and a little less than 87 per cent. amitotic.

7. Therefore the conclusion is reached that amitosis plays in this instance an important, if not the chief part in the differentiation of a definitive tissue.

8. Of the two alternatives which might be suggested, one, that unobserved epidemics of mitosis account for the facts, is not only without foundation, but is improbable; the other, that a fourfold increase in cells can be accounted for on the basis of 1 mitotic division per 1,751 nuclei involves an absurdity.

Postscript.

By an oversight I have omitted a reference to Professor Hargitt's observations on the occurrence of amitotic divisions in the development of certain cœlenterates. In his paper entitled "The Organization and Early Development of the Egg of *Clava leptostyla* Ag.," Biol. Bull., Vol. X., Hargitt says: "During the early cleavage, even up to the sixteen-cell stage, no evidence of mitosis has been found." Similar experiences were met with in studying the development of *Eudendrium* and *Pennaria*, and Professor Hargitt adds: "as facts multiply . . . cytologists will be forced to take cognizance of this form of cytogeny and give it something more than a merely incidental place in cellular activities."

Quite recently, in fact after the present paper had gone to press, I received a reprint of the memoir "On *Turritopsis* (McCrady)," *Proc. Bost. Soc. Nat. Hist.*, Vol. 33, No. 8, by Professors Brooks and Rittenhouse. These authors record the occurrence of direct nuclear divisions during the development of *Turritopsis*, and incline toward the conception of Flemming and Ziegler, that amitosis is connected with cellular specialization or degeneration, as the process is most abundant in *Turritopsis* shortly before cell boundaries disappear and the embryo becomes transformed into a syncitium. As the adult is derived from this syncitial embryo it is not unreasonable to consider the amitoses in question as developmentally significant parts of a normal cycle, as stages in the establishment of adult definitive tissues, a view supported by the evidence recorded in the preceding pages.

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January 1, 1908.

body with a series of pectinations which are continuous with ridges, separated by grooves occurring on the body of the expansion. The pectinations are not always regular and do not always project from the body. In a few cases they are lacking entirely. The handle may be round, triangular or flattened and is usually more or less curved toward the pectinated edge. In some cases the entire clasping organ may be S-shaped, one of the curves occurring in the body of the comb.

The comb-like bodies were first described and figured by Fritsch in 1879 from the Permian rocks of Bohemia. Previous writers had regarded these objects as the jaw bones of fishes or lizards but further research brought out the fact, first indicated by Fritsch, that they could not be jaws since they were entirely covered by an enamel-like substance.[1] They were found associated with the remains of *Ophiderpeton pectinatum* Fritsch and the species is defined as follows: "Stäbchen des Bauchpanzers dreimal so lang als die Wirbel, rauh! Kammplatten liegen zu wenigstens 3 Paar in der Aftergegend (?)." Fritsch regarded the "Kammplatten" as occurring in the cloacal region on account of the position in which they were found on a block of shale with portions of the pelvis of the species. "Später erhielt ich ein Stück Bauchpanzer eines Ophiderpeton, neben welchen zwei dieser rätselhaften Gebilde lagen und diess brachte mich auf die Idee, dass die Kammplatten modificirte Stäbchen des Bauchpanzers sind, welche wahescheinlich in der Kloakengegend als Hilfsorgane bei der Paarung dienten."

In 1901, when the last volume of the "Fauna der Gaskohle" was issued, Fritsch figured and described another species of *Ophiderpeton*, *O. persuadens* Fr. with the "Kammplatten" in place near the cloacal region of the specimen which was composed of over one hundred consecutive vertebræ and had a length of 15 cm. Only a portion of the tail was lost. Fritsch says in regard to this species: "Das interessanteste bei diesem Exemplar ist, dass in der Nähe des Afters zwei Rudimente von Kammplatten liegen, . . . welche meine Vermuthung bestätigen dass diese Organe in der Cloakengegend gelegen, ein Hilfsapparat bei der Paarung waren."[2]

[1] Fritsch, 1883, "Fauna der Gaskohle," Vol. I., p. 122.

[2] Fritsch, 1901, "Fauna der Gaskohle," Supplement, Vol. IV., p. 89.

there are set fine serrated tooth-like projections varying in number from fifteen to sixty.

Cope announced the discovery of similar bodies in the Linton, Ohio, beds in 1885[1] and compared them to the elements described and figured by Fritsch as "Kammplatten." He says in regard to them: "They consist of a curved rod terminating in a secund expansion, whose projecting edge is divided into fine teeth like a comb" and they "differ from those described by Fritsch, in the greater curvature of the shaft in the direction to which the teeth present. Its axis is nearly at right angles to that of the body of the bone." In this connection he refers to similar bodies found by him in the Laramie deposits of Montana to which he had given the name *Ceratodus hieroglyphus* which he later changed to *Arotus hieroglyphus*. Hay in his "Catalogue of Fossil Vertebrata of North America" retains the name under *Ceratodus*.

Cope's description of the element in question is as follows: "The dentigerous plate is thin and dense, and has the appearance of a short toothed comb with a handle. The tooth-like points

FIG. 3. Cope's *Ceratodus hieroglyphus* from the Laramie of Montana. × 4.

are the extremities of low ridges, which are arranged nearly at right angles to a wide longitudinal elevated half of the osseous base. They are separated by shallow grooves from each other and are not continuous with the basis just mentioned, which rises abruptly above them. They are smooth. The 'handle' above alluded to is triangular in section having two bevels on the side supporting the tooth ridges. The lower face of the bone is smooth.

MEASUREMENTS.	M.
Total length	.013
Length of dentigerous portion	.010
Total width	.0045
Width of dentigerous portion	.0020

"There are thirteen teeth in the length"[2] (Fig. 3).

[1] Cope, 1885, *Pal. Bull.*, No. 40, p. 405.

[2] Cope, 1876, *Proc. Acad. Nat. Sci. Phila.*, p. 260.

Through the kindness of Dr. L. Hussakof of the American Museum, I have had the opportunity to examine the object described by Cope and am able to verify his description. The fragment, which lacks the greater part of the handle, is of a reddish brown color and is covered with a shining enamel very similar to that described by Fritsch for the clasping organs of *Ophiderpeton*. The object has very little resemblance to a *Ceratodus* tooth, as may be seen by referring to the figure herewith given (Fig. 3).

In the Laramie deposits remains of five species of Amphibia have been discovered. The forms, the fragments of which are rather abundant in the deposits, are based on small portions of the skeleton which are typically amphibian in structure. The forms described from the Laramie Cretaceous of North America are: *Scapherpeton tectum* Cope, based on a single vertebra with a fragment of another bone; *S. laticolle* Cope, based on several vertebræ and a limb bone; *S. excisum* Cope, based on several vertebræ; *S. favosum* Cope, based on a single vertebra, and *Hemitrypus jordanianus* Cope, based on a single vertebra.[1] Lambe has figured vertebræ of *Scapherpeton tectum* Cope, and provisionally referred fragments of a jaw to this species from the Judith River beds of Canada.[2]

Cope suggests[3] that the comb-like bodies described above may have been the clasping organs of the *Scapherpeton* forms of the Laramie. The element described as *Ceratodus hieroglyphus* Cope, certainly agrees in every detail with the amphibian elements figured and described by Fritsch and Stock and there can be no doubt that it is not a fish tooth but is probably the clasping organ of some one of the Laramie Amphibia. If it is the clasping organ of some one of the Laramie amphibians certain interesting deductions would necessarily follow. Among the earlier forms the clasping organs are only known among some of the Carboniferous Microsauria and Aistopoda. If these organs are present in the Laramie forms and also in the Carboniferous forms there must be some genetic relationship between them. *Hylæobatrachus* from the Wealden of Europe does not

[1] Cope, 1876, Proc. Acad. Nat. Sci. Phila., pp. 353–359.
[2] Lambe, 1902, *Contrib. Canad. Paleon.*, Vol. III., Pt. II., p. 31.
[3] Cope, 1885, *Pal. Bull.*, No. 40, p. 408.

exhibit, so far as I am aware, any evidences of the clasping organs and this is the only form which is intermediate in age between the Carboniferous forms and the Laramie ones. The Labyrinthodontia which existed in the Permian and Trias are highly specialized and so cannot be taken into account in this regard. Newberry,[1] however, was strongly inclined to the idea that the "Kammplatten" were in reality fish teeth as Barkas and Traquair had thought. He says it will take strong evidence to convince him of the fact that they are amphibian. He mentions the discovery of several of the "Kammplatten" in the Linton beds and says they differ but little from those described by Fritsch.

Fortunately the exact location of those objects in the anatomy of the extinct amphibians is not a matter of conjecture, but of actual knowledge, since Fritsch has discovered them in place on the specimen of *Ophiderpeton persuadens* Fr. above referred to. That they are but modified elements of the ventral armature is also beyond a doubt since Fritsch found intermediate forms of the ventral chevron rods and figured several of them. A copy of one of these rods is given in Fig. 4. The abdominal arma-

FIG. 4. A slender, toothless clasping organ from the Permian of Bohemia. After Fritsch.

ture is almost universal among the Carboniferous forms of the Amphibia, being unknown in a few forms such as *Pelion lyelli* Wyman and *Molgophis macrurus* Cope, so that we may expect to learn of forms which had the clasping rods developed and of which there is now no knowledge. What purpose the abdominal chevron rods served is not at present apparent but that the posterior rods near the cloacal region became specialized into clasping organs for the retention of the female during the breeding season seems almost beyond dispute.

Since the clasping organs are thus shown to be but specialized

[1] Newberry, 1889, Monograph, U. S. G. S., Vol. XVI., p. 228.

parts of the abdominal armature it is to be expected that the forms of the Amphibia which lived during Laramie times would have the armature well developed as they almost certainly had the clasping organs. When the Laramie Amphibia are better known they will, without doubt, be found to have at least some representation of an abdominal armature.

How these clasping organs served the purpose for which they seem to be developed is, in large part, a matter of conjecture since we do not know and in all probability never can know the method of copulation in the extinct forms. It is a matter which is well known that the male of the common newt retains the female by means of roughnesses developed on the inner side of the hind limbs and why may not the clasping organs, above described, have served the extinct forms in a similar manner? Of course it is readily seen that they would be of more service to the limbless forms like *Ophiderpeton* than they would to forms like *Scapherpeton* which, in all probability, had well developed limbs. It is possible that the clasping organs in *Scapherpeton*, if they belong to this form, were vestigial and were not functional or they may have belonged to limbless forms of which there is no knowledge unless *Hemitrypus* is a limbless amphibian.

So far as I am aware, the clasping organs are found in association with limbless forms only. Such was the case in all of those described by Fritsch. The form of limbless amphibian described by Huxley[1] from the Coal-measures of Ireland seems not to have had the clasping organs preserved or at least they have not been detected. The "Kammplatten" discovered by Stock, Barkas and Traquair in the rocks of England were not associated with other remains so we do not know to what type of amphibian they belonged, although a species of *Ophiderpeton*, *O. nanum* Hancock and Atthey, has been described from this region. In the Linton, Ohio, deposits there occur a number of forms which are limbless. The species of *Ptyonius*, *Molgophis*, *Hyphasma*, *Phlegethontia* and possibly a species of *Osetocephalus* also was limbless, although there would seem to be indications of limbs in the other species. In any case the first four probably do not possess limbs and in *Molgophis*, apparently, the abdominal rods

[1] Huxley, 1867, *Trans. Roy. Irish Acad.*, Vol. XXIV., p. 353.

were not developed so that clasping organs may safely be ascribed to species of the other three genera. There is no reason why the limbed forms might not also have possessed the clasping organs.

Among the recent Amphibia, so far as I am aware, there are no bony or cartilaginous elements in the abdominal wall. Both the chevron armature of the extinct forms and the well developed " Kammplatten " are absent among the modern Amphibia. The recent forms do, however, have well developed clasping organs, at least in some cases. It will be of interest, in this connection, to examine the condition of the clasping organs in the recent forms as a comparison to those found in the extinct species.

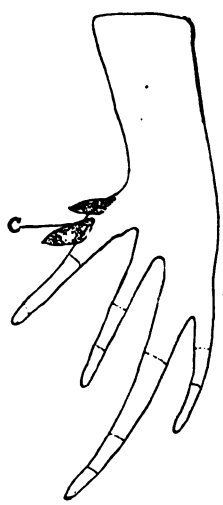

FIG. 5. The right hand of *Leptodactylus*. From a specimen in the Field Museum. Enlarged.

Among the Salientia the clasping organs are usually developed on the fore limbs and consist for the large part of wart-like or spine-like excresences on the skin. These asperities are developed on the inner side of the fore legs and on the breast of the males. " Nuptial excrescences on the inner metacarpal tubercle and on the inner fingers of the male are common ; they reach their greatest development in the Himalayan *Rana liebigi*, the male of which is ' remarkable for the extreme thickness of its arms, the inner sides of which are studded with small conical black spines, each supported on a rounded base produced by a swelling of the skin. A large patch of similar spines exists on each side of the breast.' " [1]

In the genus *Leptodactylus*, from Central and South America, a specimen of which I have studied in the Field Museum, the first digit is somewhat swollen to support two black, horny spines (Fig. 5), which project on the inner side of the finger. There are many variations of these two extreme cases cited above but in all they are on the same general plan. In most cases the clasping organs are only developed during the breeding season.

[1] Boulenger, " Cat. Batrach. Salientia," p. 22. Gadow, 1901, " Amphibia and Reptiles," p. 250.

Among the Caudata the clasping organs are almost always developed on the hind limbs. They were first observed in the common newt, according to Jordan,[1] by Braun in 1878 on the European species *Diemyctylus alpestris*. During the breeding season there are developed "all the way up and down the inside of the hind legs as well as on the adjoining parts of the body, round, black, wart-like elevations. These warts are hard and rough and undoubtedly aid the male in clasping the female more firmly."[2]

There are numerous other instances cited in the literature in which similar structures have been observed in the males but they are all in general plan similar to those of *Diemyctylus* where they are only developed during the breeding season and subsequently turn yellow, become soft and then disappear.

The phylogeny of the modern Amphibia is one of the most obscure of any of the groups of vertebrates. The modern forms are for the most part degenerate in structure and in no way compare to the robust forms of the Carboniferous, some of which, at least, must have been their ancestors. The Amphibia on the whole have played but a small part in the history of animal life on the earth. They have never become the dominant type in any age as did the fishes, reptiles and mammals. They have always, so to speak, filled in the corners, left by their more aggressive contemporaries. Their chief interest lies in that they were the ancestors of the higher forms of life.

The modern Amphibia are for the most part shy, harmless creatures although there is an interesting exception to this in the case of the horned frog, *Ceratophrys*, one species of which, according to Lydekker, is "exceeding bold and ferocious, flying fiercely at anyone who attacks them, and maintaining their hold with the tenacity of a bull-dog, at the same time uttering a kind of barking cry."[3] We have abundant evidence that in the old pond or lake which was once located, during the Carboniferous period, near the place where was recently situated the town of Linton, Ohio, that the Amphibia, like the recent *Ceratophrys*, were of a

[1] Jordan, 1891, *Journ. Morphol.*, Vol. V., No. 2, p. 263.
[2] Jordan, *loc. cit.*, p. 264.
[3] Lydekker, "New Natural History," Vol. V., p. 275.

ferocious disposition. They were well fitted for such a life armed as they were, in some cases, with long, strong teeth and hard dermal plates and scales. There is an abundance of evidence to their carnivorous habits in the coprolites preserved with their remains. As some of the forms of the Carboniferous may have reached a length of some ten to twelve feet they would be ferocious creatures to attack in comparison to the modern degenerate forms. The suggestion as to the evolution and ancestry of at least one group of the modern Amphibia will be given elsewhere but the rocks have not yielded, as yet, a great amount of information which might serve to connect the old and recent forms.

The earliest geological evidence of Amphibia are the foot-tracks described by Lea in 1849, and subsequently made more fully known by Marsh, from the Catskill Formation, Upper Devonian, of Pennsylvania. The next evidence is that of abundant remains of amphibians from the Allegheny series of the Pennsylvanian in North America and in probably equivalent strata in Europe. Abundant remains are known, also, from the Permian of North America and Europe. Fritsch and Credner, especially, have described abundant faunæ from the Permian rocks of Bohemia and Saxony and Cope has done the same for the Permian beds in North America. In the Triassic the majority of the Amphibia are the highly specialized stereospondylus labyrinthodonts, although a few smaller and more primitive forms are known. The next evidence of Amphibia is the discovery by Dollo of a complete skeleton of a perennibranchiate salamandrine form from the Wealden of Bernissart.[1] From *Hylæobatrachus* in the Wealden to the forms described by Cope from the Laramie Cretaceous of Montana our knowledge of the Amphibia is a blank. The forms must have existed somewhere but their remains have not yet been discovered. Marsh, it is true, gave a name to some fragments from the Lower Cretaceous of Wyoming but these he never figured and never described and so far as our knowledge of the form goes the name *Eobatrachus agilis* Marsh may be considered as a mere *nomen nudum*. In the Eocene rocks, remains of Amphibia are fairly abundant but they represent types which compare with the modern forms in structure and in no way

[1] Dollo, 1884, *Bull. Mus. Roy. Hist. Nat. de Belg.*, III., p. 85.

resemble the ancient species. In the Miocene rocks of Switzerland occurs the famous *Andrias scheuchzeri* Tschudi, which is related to the Japanese salamander of to-day. In the Pleistocene are found remains which belong to species of modern genera.

That the transition between the ancient and the recent forms took place between the close of the Permian and the close of the Cretaceous is evident. This transition consisted in the loss of the ventral armature, the loss of sclerotic plates, the loss of dermal plates, the loss of the bony clasping organs, the loss of the supra-occipital and epiotic elements from the skull and the loss of strong teeth. The modern Amphibia are degenerate in structure. The bridge which may help to close the gap between the Paleozoic and the Mesozoic forms is the fact that similar bony clasping organs are, apparently, developed in the forms of the two periods. If this be true it will be of service in closing one of the widest gaps in vertebrate phylogeny.

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BIOLOGICAL BULLETIN

THE DEVELOPMENT OF HYDRA.

GEO. W. TANNREUTHER.

INTRODUCTION.

The development of hydra has been described by Kleinenberg (4), Kerschner (5), Brauer (3), and a few others, yet there are many points of interest and importance that have not been presented, especially in the origin and formation of the ovary, in fertilization and in early cleavage.

The following results are based mostly upon one species: *Hydra* sp.? (Brauer) or *H. dioecia* (Downing). The three forms *H. viridis, H. grisea and H. fusca* are universally recognized as distinct species. *Hydra* sp.? (Brauer) has been observed by many investigators, yet some prefer to consider it as a variety of *H. fusca.* Brauer at first placed it with *H. fusca*, but upon discovering that the sexes were separate, designated it as *Hydra* sp.?. Downing (2) in his paper on "Spermatogenesis of *Hydra*" uses the name *H. dioecia.*

Hydra sp.? (Brauer) which varies in color from a light to a dark brown, resembles *H. fusca.* When we take into consideration, however, that the sexes are separate, that the eggs are glued to the object on which the parent rests by a secretion from the ectoderm, and that the embryo may hatch out while the egg is still attached to the parent form, it is perhaps justifiable to regard it as a distinct species.

ORIGIN AND FORMATION OF THE OVARY.

The early formation of the ovary differs from Kleinenberg's account as it is usually given in text-books. The interstitial cells, which divide with nearly the same rate over the body of

the hydra, are about uniform in size, shape and appearance. The formation of the ovary first becomes recognizable by a rapid growth of the interstitial cells in some special region and not by their more rapid division. After these cells have increased several times in volume, they become differentiated into two distinct regions, namely, a more central region, which gives rise to the ovum or ova, and a peripheral region which may be considered the temporary ovary, whose cells cease to enlarge and later contribute directly to the formation of the yolk (Pl. VIII., Fig. 2). The ectodermal cells, with their scanty supply of cytoplasm and nuclei that stain very faintly, are pushed more to the exterior by the enlarged interstitial cells. They often remain connected with the mesoglea by fibrous strands.

The cells of the central region vary in number and their contents contribute directly to the formation of the ovum or ova (Fig. 2, *a*, *b*, *c*). Not merely one but all of these cells continue to enlarge. This increase in size affects the nuclei as well as the cell bodies. The cell walls break down and the cytoplasm which now becomes a common multinucleate mass without any definite outline comes to lie between the enlarged cells of the peripheral region of the ovary and the mesoglea (Fig. 3). The egg at this stage of development as stated above is multinucleate. All of the nuclei enlarge somewhat, the chromatin assumes the spireme condition and the nucleoli are very prominent. One of these nuclei, seldom more than one (Fig. 3, *a*), continues to enlarge and becomes the egg nucleus. The remaining nuclei gradually break down and disappear within the cytoplasm. When two nuclei persist, the cytoplasm becomes separated into two distinct parts. Each part with its contained nucleus becomes a separate egg, which develops independently of the other. The egg does not begin as a single cell, but as a multinucleate mass which results from the fusion of several cells after the breaking down of their walls. Thus, the cytoplasm which originates from several cells becomes the cytoplasm of the egg. Up to this stage of development it is very difficult to determine the exact origin of the sexual organs except from a study of sections. The ovaries and spermaries both begin by a rapid growth of the interstitial cells, but in the formation of the spermary, when

ovary are taken up by the amœboid egg and become changed into the yolk or pseudo-cells of the egg. Fig. 6 represents a cross-section of several pseudopodia into which the nuclei of the interstitial cells of the ovary are passing. The transformation of these interstitial cells into yolk is shown in Fig. 7, *a–c*. The chromatin becomes very granular and forms a band around the inner border of the nuclear membrane. The nucleolus becomes imbedded in this band of granular chromatin and the nucleus has the appearance of a hollow sphere with its wall thickened on one side. After the yolk or pseudo-cells are formed they divide amitotically.

According to Kleinenberg, the interstitial cells of the ovary surrounding the egg break down and act as food for the developing egg. Brauer says the interstitial cells, after breaking down, enter the egg and give rise to the pseudo-cells.

After the amœboid egg becomes filled with yolk, the pseudopodia are drawn in and the egg becomes nearly spherical (Pl. XI., Fig. 33, *a*), and is surrounded by a single layer of ectodermal cells except at its base. The egg nucleus during the contraction of the pseudopodia becomes very faint and is difficult to recognize. According to Brauer, the nucleus becomes entirely invisible.

Abortive ova are often found in sexually reproducing hydra. They consist of a small mass of yolk cells surrounded by a thin egg membrane, and are devoid of a nucleus. The ovary begins as in the normal cases, but instead of one of the nuclei persisting in the multinucleate cytoplasmic cell mass, they all break down, leaving the common mass of cytoplasm without a nucleus. No pseudopodia are formed. This condition would be represented in Pl. VIII., Fig. 3, if no nuclei were present in the cytoplasm between the enlarged interstitial cells and the mesoglea. Some of the nuclei of the interstitial cells enter the cytoplasm as in the normal egg and form the yolk. The common mass of cytoplasm with its contained yolk now becomes spherical. The abortive ova do not break through the ectoderm, but are gradually absorbed.

Maturation.

Immediately after the pseudopodia are drawn in, the polar bodies are formed (Pl. XI., Fig. 33, *a*, *pb*). When formed they

point where the sperm enters the egg (Brauer). The egg becomes surrounded by a number of sperm, several of which may pass into the egg membrane, but only one enters the egg. It is interesting to note that the egg may remain susceptible to the sperm twenty-four hours after it passes through the ectoderm, but if the sperm are not added within twenty-four to thirty hours after maturation or the passing of the egg to the exterior, fertilization will not take place.

Two lots of hydras with eggs, one twenty-four hours and the other immediately after maturation, were placed in separate vessels containing water and sperm added. Fertilization occurred in both instances within two hours. The rate of cleavage was similar in both cases. In the unfertilized eggs, the yolk spheres cease dividing, gradually break down and the egg becomes a hollow membranous sphere containing a fluid substance.

Almost immediately after fertilization the peripheral cytoplasm becomes free from yolk and is more finely granular than the cytoplasm within the egg.

The entrance of the sperm and union of male and female pronuclei agree with Brauer's account. After the union of the pronuclei the cleavage nucleus passes a short distance into the egg from the animal pole and divides (Pl. IX., Fig. 9).

Cleavage.

The cleavage of *Hydra* sp.? is total, unequal and regular. Brauer states that the cleavage is equal and total, but he gives no figures to show the early cleavage stages. Kleinenberg says the cleavage (*H. viridis*) takes place in a remarkable manner and that pseudopodia or cleavage papillæ are formed at the point where the first cleavage begins. He also describes the second cleavage as very erratic, and states that the egg undergoes peculiar changes during cleavage.

The cleavage of *Hydra* sp. ? does not exhibit such erratic conditions as Kleinenberg describes for *H. viridis*.

Just before the first cleavage the egg changes from a spherical to a more oblong form, and the cleavage passes through the short axis. This peculiarity is true only of the first, second and third cleavages, being especially well marked in the first and second.

from side view in the living material. The third cleavage furrow which passes entirely through the egg instead of becoming obliterated within, as in the first and second cleavages, becomes the cleavage cavity. Pl. IX., Fig. 13 represents a section passing through the poles of the egg shortly after the third cleavage is complete. The cleavage cavity is very distinct and shows the nuclei near the inner ends of the cells. The egg now becomes more spherical and the inner ends of the cells become rounded off. The blastomeres of the vegetative pole are larger than those of the animal pole. The fourth and fifth cleavages are parallel to the third, but there is some irregularity in their time of formation. Both cleavages may begin at the same time, but in most instances observed the cells above the equator divided first. They differ from the first, second and third cleavages in that the cleavage does not start at one side and gradually pass to the opposite, but instead begins at different points on the surface at the same time. The cleavage furrows are very indistinct. Pl. X., Fig. 14 represents the fourth and fifth cleavages completed, as they appear in a plane passing through the poles. The cleavage cavity is very irregular and increases considerably in size (Pl. IX., Fig. 15) without any further division of the cleavage cells. This peculiarity is due to a change in the form of the cells, whereby their long axis becomes really tangential. The cleavage cells now divide very rapidly and it is impossible to distinguish any further regularity in the process of cleavage (Fig. 16).

Origin of Endoderm.

When the cleavage cavity reaches its maximum growth, the embryo consists of a large spherical blastula with its single layer of primitive ectodermal cells, which have about the same thickness throughout; but some of them now begin to enlarge, so that they come to project into the cleavage cavity (Pl. X., Fig. 17), and instead of dividing parallel to the surface begin to divide at right angles to it. The inner ends of the divided cells become free in the cleavage cavity and give rise to the endoderm. A section passing through the equatorial plane (Pl. IX., Fig. 18) shows the cells not only dividing radially but also tangentially. This process continues until the cleavage cavity becomes filled with cells

(Fig. 19). The cells within the cleavage cavity also divide very rapidly (Pl. X., Fig. 20), and the embryo becomes a solid spherical mass of cells with the cleavage cavity entirely obliterated.

The formation of the endoderm begins uniformily at the different poles of the blastula. Its origin is multipolar as Brauer states. According to Brauer, during the formation of the endoderm some of the cells which divide radially and remain within the wall of the blastula force the primitive ectodermal cells, which have a narrow base, from the periphery into the cleavage cavity before they divide, and thus an entire cell of the primitive ectoderm becomes an endodermal cell. The species studied showed a number of cells dividing radially in the periphery during the formation of the endoderm, but I was unable to find any indication that entire cells were forced from the periphery into the cleavage cavity.

According to Kerschner (5) and Korotneff (6), the endoderm is formed by the inwandering of cells from the vegetative pole of the egg.

When the endoderm is completely formed division ceases and the endodermal cells with their abundance of yolk can readily be distinguished from the cells of the outer layer or ectoderm.

Egg Membranes.

The outer and inner egg membranes are formed from the ectodermal cells. The outer membrane begins as an outgrowth from the different cells of the ectoderm. A very small portion or nearly all of an ectodermal cell may take part in this process (Pl. X., Figs. 20–22). These outgrowths in the early formation of the membrane remain continuous with the cell from which they originate, and are often nearly as large as the body of the cell itself (Fig. 23, *o*). The outer protoplasmic ends of these projections assume various shapes (Fig. 25). The thin elastic wall surrounding these outgrowths, which is a continuation of the wall of the ectodermal cell, often becomes very delicate, breaks through and allows the cytoplasm of the cell to flow out, thus causing the cell to collapse. The different outgrowths now fuse at their basal ends and form a continuous membrane around the developing embryo (Fig. 25). This membrane becomes very tough and has

the nature of chitin. When it is nearly formed, a second or inner membrane begins as a secretion from the ectodermal cells over the entire embryo just beneath the outer egg membrane (Figs. 25 and 26).

According to Kleinenberg, the entire primitive ectoderm is used in the formation of the outer and inner membranes. The formation of these membranes in *Hydra* sp.? confirms Brauer's account in that the inner ends of the ectodermal cells persist and become the definitive ectoderm.

After the membranes are formed, the eggs are glued to the object on which the parent rests. This, however, is not always true. In a few instances observed, the embryo hatched out while the eggs were yet attached to the parent. Brauer states that the eggs are glued by a sticky secretion from the ectoderm to the object on which the parent rests, and that the parent remains in contact with the egg until the embryo hatches out.

The formation of the interstitial cells and of the body cavity was not studied. The embryo hatches out in from eight to ten days after the outer and inner membranes are formed.

General Remarks.

The condition necessary for the appearance of sexual organs in hydra has long been a question of much interest, especially among scientific investigators. Various chemical solutions as well as different conditions of food and temperature have been tried with little or no success.

Downing (2) by subjecting hydra to various degrees of reduced temperature was able to get hydra to produce sexual organs after an exposure in a dark refrigerator to a temperature of about 12° C. for twelve hours. But as sexual organs appeared at the same time on several control hydras in the laboratory which were kept in the light at the temperature of the room, he concluded that light and temperature are not controlling factors in determining the appearance of sexual organs.

During the three different years that Hydra sp.? were collected at intervals from two to three times a week, no sexual organs were found, but buds were abundant in the winter season as well as during the warmer months. From this I was led to infer that

dermal and endodermal cells were similar to those in hydra during the formation of the sexual organs. This would indicate that lack of food and production of sexual organs give similar effects. Sexual organs never appeared on well-fed hydras, except those that had passed through a vigorous process of budding for a definite time. This indicates very forcibly that food is not a direct controlling factor in the production of sexual organs, but instead favors vigorous budding, which in turn gives rise to conditions that cause the appearance of these organs in the animal's life history. The question immediately arises, what are these conditions? Is it some inherent factor within the interstitial cell that has to do with the appearance of the sexual organs irrespective of food, temperature, etc.; or is it perhaps the inability of the cells in general to assimilate the food present after an active process of budding which leads to the differentiation of sexual organs in some definite region so that a new cycle may be started? The latter view seems the more plausible, as the ectodermal cells do pass through a marked change in those hydras that have been budding actively for some time. The cells become less granular, numerous vacuoles appear, the nuclei stain less intensely and very seldom divide. These conditions, however, vary in different parts of the same hydra. Moreover, as the ectodermal cells show this somewhat degenerate or exhausted condition in the parent hydra that is actively feeding and budding, it suggests very forcibly, as stated above, the inability of the ectodermal cells to assimilate the food present.

SUMMARY.

The interstitial cells which give rise to the ovary, after increasing in volume become differentiated into two distinct regions: a central region which contributes directly to the formation and growth of the ovum, and a peripheral region whose nuclei later enter the egg and become changed into yolk.

Occasionally two nuclei persist in the central region and give rise to two distinct ova. Each ovum has its individual membrane and is entirely independent of the other. The ova are forced through the small opening of the ectoderm to the exterior by the contraction or pulling back of the ectoderm. The polar bodies

remain attached to the egg until after the third cleavage by means of a connecting thread of cytoplasm. When the eggs are not fertilized they disintegrate and finally fall to pieces.

The cleavage is total, unequal and regular. The cleavage cells communicate with each other by means of protoplasmic bridges or connections. There is a passage of substance from one cleavage cell to the other. The cleavage cavity begins with the third cleavage. The blastula when completely formed is a hollow sphere of primitive ectodermal cells.

The origin of the endoderm is multipolar. The outer and inner egg membranes are formed from the ectoderm; the first is formed by an outgrowth of the ectodermal cells, the second by means of a secretion from the same cells. The inner ends of the ectodermal cells persist and become the definitive ectoderm. The gastrula consists of a solid spherical mass of cells surrounded by the egg membranes. The eggs are either glued to the object on which the parent rests or remain attached to the parent until the embryo hatches. The eggs of *Hydra* sp.? will not continue to develop when removed from the parent after fertilization occurs, or even after cleavage has begun.

Hydras seldom continue to reproduce by budding after the sexual generation is completed. Exhaustion due to vigorous budding precedes the appearance of the sexual organs, more especially the ovaries.

Hydra sp.? reproduces by budding during the entire year. No sexual organs were found on the hydras when collected, but after the animals had been kept in aquaria with abundance of food, sexual organs appeared on those hydras that had been budding vigorously for several weeks. Spermaries or ovaries never appeared on buds.

It gives me pleasure to express my gratitude to Dr. George Lefevre for reading this manuscript.

Zoölogical Laboratory,
University of Missouri,
January 30, 1908.

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EXPLANATION OF PLATE VIII.

FIG. 1. Egg with polar bodies, immediately after it has passed to the exteri the ectoderm. *e.m.*, egg membrane.

FIG. 2. Section showing early formation of ovary at the time the interstitial become differentiated into two distinct regions; *a*, *b*, and *c*, cells of the ce region that are directly concerned in the formation of the ovum or ova; *p.r*, cel the peripheral region which contribute to the formation of the yolk. × 76.

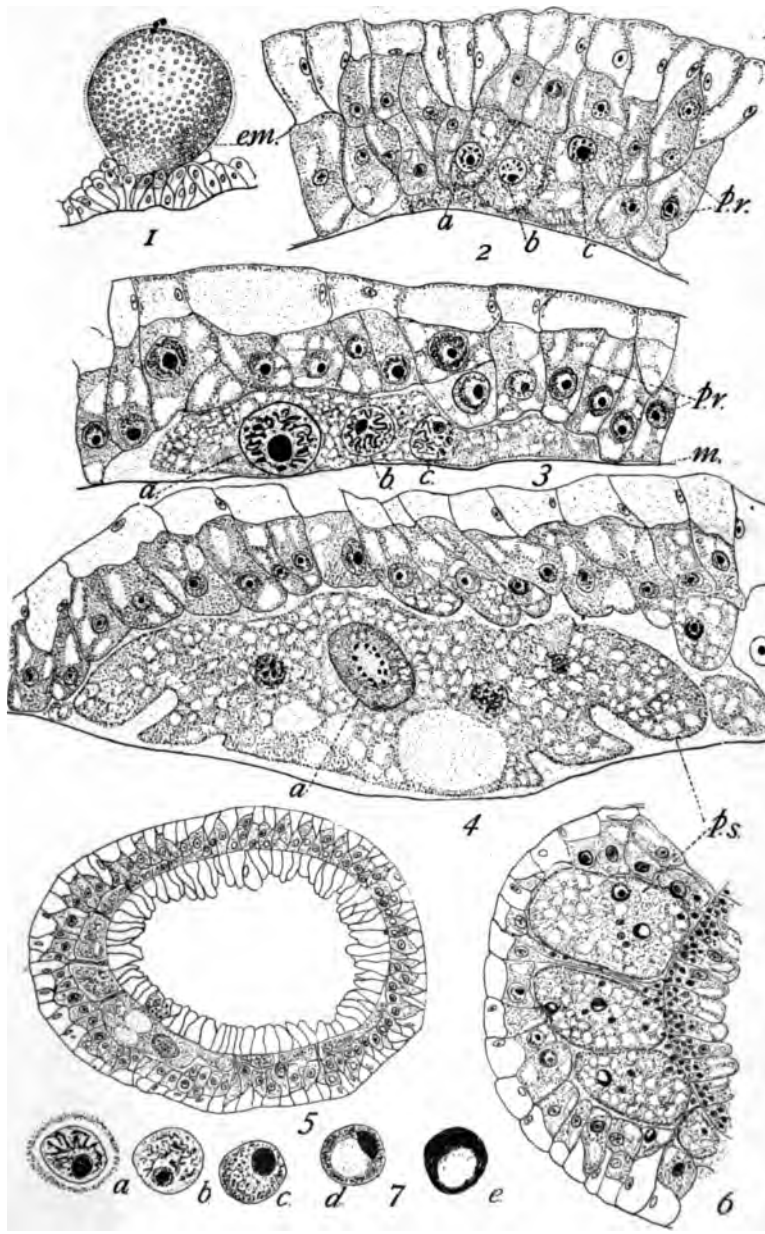
FIG. 3. Section, little later than preceding, showing the ovum between the i stitial cells, which later become the yolk, and the mesoglea; *a*, nucleus, w becomes the egg nucleus; *b.c*, nuclei breaking down within the egg cytoplasm mesoglea.

FIG. 4. Section of ovum showing the formation of the pseudopodia; *a*, nucleus; *p.s*, pseudopodia. × 80.

FIG. 5. Cross-section of ovary, little later than preceding, just before the form of the yolk or pseudo-cells.

FIG. 6. Cross-section of several pseudopodia showing the entrance of nucl the interstitial cells of ovary, which become the yolk.

FIG. 7. *a–e*, five stages in the transformation of interstitial nuclei into yolk.


em.
1
a
2
b
c
p.r.
p.r.
m.
3
a
b
c
a
4
p.s.
5
a
b
c
d
7
e
6

EXPLANATION OF PLATE IX.

FIG. 8. Section through the animal pole of egg showing polar bodies with connective thread of protoplasm; *p.b*, polar bodies; *pr.n*, pro-nucleus; *e.m*, egg membrane. × 750.

FIGS. 9 and 10. Sections of eggs passing through poles, showing first cleavage and protoplasmic connections between cleavage cells: *p.c*, protoplasmic connections.

FIG. 11. First cleavage completed, and division of nuclei for second cleavage. × 55.

FIG. 12. Section of egg at right angles to polar axis, showing second cleavage complete. × 58.

FIG. 13. Section of egg passing through poles with third cleavage completed and beginning of cleavage cavity; *cl.c*, cleavage cavity.

FIG. 15. Fourth and fifth cleavages complete. Cleavage cells becoming flattened out around the cleavage cavity.

FIG. 16. Stage a little later than preceding.

FIG. 18. Maximum development of cleavage cavity; primitive ectoderm cells dividing to form endoderm.

FIG. 19. Cleavage cavity becoming filled with endodermal cells.

EXPLANATION OF PLATE X.

FIG. 14. Fourth and fifth cleavages completed; *a*, animal; *v*, vegetative pole.

FIG. 17. Blastula with primitive ectoderm cells, just before the formation of the endoderm.

FIG. 20. Cleavage cavity completely filled with endoderm cells and the beginning of the outer egg membrane.

FIGS. 21–26. Formation of the outer and inner egg membranes; *o*, outgrowths.

FIGS. 27–32. Different shapes which the egg assumes in passing to the exterior of the ectoderm.

EXPLANATION OF PLATE XI.

FIG. 33. Longitudinal section of aboral end of hydra, showing eggs in different stages of development; *e.c*, ectoderm surrounding egg; *p.b*, polar bodies; *prn*, pronucleus; *e.m*, egg membrane.

FIGS. 34–36. Surface views of living eggs, showing first cleavage; side view; *p.c*, protoplasmic connections; *p.b*, polar bodies.

FIGS. 37 and 38. Second cleavage, side view.

FIG. 39. Polar view of second cleavage.

FIGS. 40–42. Third cleavage of living egg; side view; *p.b*, polar bodies.

ation to that of second regeneration, age and level of injury being the same. Two comparisons were made and in the averages second regeneration is slightly less than first in one case and equal to it in the other. First regeneration .35, second .33; first .26, second .26. It is to be concluded from the averages that second regeneration differs very little from the first.

3. *Location of Injury.* — The effect of location was obtained from a set of tadpoles that had had 10 mm. of tail removed. After they had regenerated about 5 mm. they were again operated upon — one half 3 mm. caudad of first cut and the other half 3 mm. cephalad of the first cut. The first half gave a regeneration from tissue but recently laid down, the second a regeneration from old tissue in a tadpole that had just been regenerating from a level nearer the tip of the tail. Neither half varied greatly from the control but both were below it. The averages for those cut caudad of first injury are .23, control .24; .18, control .21; for those cut cephalad .41, control .46; .33, control .34. The difference here as in the comparison of first and second regeneration is in favor of the previously uninjured animal. It is not striking however, and shows that regeneration in either of the two cases presented by the two halves of this set, is almost the same as the first regeneration from the same level.

4. *Level of Injury.* — The rates of regeneration of tadpoles with 7, 10, and 13 mm. of tail removed were compared. From their simple rates it was quite evident that the rate of regeneration in the tail is directly influenced by the amount of tail removed. This is in direct accord with the work done by Spallanzani, who found the whole leg of a salamander to regenerate as soon as a part of it, and the more recent work of Morgan[1] on the tail of *Diemyctylus* in which he states, "the nearer the cut to the outer end the slower the rate of regeneration." However, in order to state this change in rate more concisely, the rate of regeneration was divided by the amount of tail removed and a "proportional rate" obtained. From this proportional rate it was found that the rate of regeneration varies not only directly but *proportionally with the distance the cut is removed from the tip of the tail.*

This point of proportional regeneration is now being worked up in detail in connection with the influence of age.

[1] *Jour. Ex. Zoöl.*, Vol. III., No. IV., 1906.

Two sets of tadpoles of different ages were used so the data given in the table below are divided into two parts.

Level of Cut in mm.	Rate of Reg.	Prop. Rate.
7	.24	.034
10	.34	.034
13	.43	.033
7	.19	.027
10	.26	.026
13	.34	.026

SUMMARY.

1. The rate of regeneration of a tadpole tail varies inversely as the age of the tadpole.

2. In successive regenerations from a given level the first and second regenerations are approximately equal.

3. Regeneration from recently regenerated tissue, and from old tissue of a tadpole tail that has been regenerating at a level nearer the tip of the tail, is almost the same as first regeneration from the same level.

4. The rate of regeneration in the tail of tadpoles is directly proportional to the distance of the level of the cut from the tip of the tail.

NOTES ON THE BREEDING HABITS OF AMBLYSTOMA PUNCTATUM.

ALBERT HAZEN WRIGHT.

Probably in early spring no amphibian eggs are more common in central New York than those of *Amblystoma punctatum*. And yet our knowledge of the salamanders themselves, from their first appearance at this season through the egg-laying period, is meager as compared with what is known subsequent to the deposition of their eggs. Of the early part of the breeding period I wish to record some notes made at Ithaca by various members of the Department of Neurology and Vertebrate Zoölogy during the last eight years.

Ithaca is located at the south end of Cayuga Lake valley. North and south of the city are large swampy areas. On the east, south and west are high steep hills, through which are cut numerous ravines. In these places and in marshy areas on the hills, *Amblystoma* is found to be very abundant in early spring.

At this season the salamanders migrate from winter quarters to suitable breeding-places, and the Ithaca marshes have always proved a favorite locality. Along the borders and through the middle of these swamps are several steam railroads and one electric railway. These prove an excellent check on the first appearance of *Amblystomas*, toads, wood-, meadow- and pickerel-frogs, and were it not for these railroads our records of first appearance would coincide with those of egg-laying.

In one place, where the electric railway passes near the mouth of a large ravine, 100–150 of *Amblystoma punctatum* are killed yearly. On April 6, 1906, 54 were counted, all having been killed the previous evening. The migration does not begin until dark. The street cars run until 11 P. M. and cross this spot about thirty times in an evening. When, as above stated, 54 were killed in four hours, what must be the number that cross these tracks during these intervals and after 11 P. M.!

The migration is quite clear. In the ravines in early spring we obtain the adults, but never their eggs. At the time the

salamanders cross the railway tracks, or a day or two subsequently, we record spermatophores and eggs in the ditches and swamp just beyond the track. Many individuals are slightly injured, and these we often find under cover near by. They almost invariably have bruised heads.

In equal abundance this form may be taken along the other borders of this swamp. From another region living individuals are secured in considerable numbers. As many as fifteen have been taken here within one hour, nor is it unusual to secure as many over the same area, two or three days later. During the day the salamanders crawl under the logs and loose railroad ties which lie along either side of the railroad embankments. A rake usually is employed, for it is under the ties partially submerged in the water that we obtain the largest number of individuals. At the southwest corner of Cayuga Lake they are found to be common under the leaves at the foot of the high perpendicular rock walls.

Three of our amphibia appear almost simultaneously: the spotted salamander (*Amblystoma punctatum*), the peeper (*Hyla pickeringii*), and the woodfrog (*Rana sylvatica*). If there is any difference or succession it is indicated by the order in which they are named. During the last eight years these species have appeared in spring as follows:

Amblystoma punctatum.	*Hyla pickeringii.*	*Rana sylvatica.*
1900, April 6	April 19	April 14
1901, April 13	April 12	April 13
1902, March 25	March 28	April 4
1903, March 13	March 15	March 19
1904, April 1	April 3	April 5
1905, April 1	March 29	April 2
1906, March 28	April 6	April 6
1907, March 24	March 25	March 28

From the above it appears that *Amblystoma* in six of these eight years preceded the peeper and the woodfrog.

The first record for 1902 was based upon twenty specimens taken by Professor H. D. Reed, from under leaves along the base of the perpendicular rocks of the west shore of the lake. "They were all found in groups of two each and proved, with one or two exceptions, to be male and female."

Whether a preliminary courtship similar to that recorded for the spotted newt (*Diemyctylus viridescens*) obtains with *Amblystomas* has always been problematic. Clarke[1] says his captive "males showed no inclination to clasp the females, but quietly deposited quite large masses of an apparently rather thick liquid, opaque white, on the bottom of the dish in which they are kept." Smith[2] remarks that "in *Amblystoma* as in axolotl there is evidently no clasping of the female by the male such as occurs in *Triton* (*Diemyctylus*)."

Mr. A. A. Allen secured at Buffalo, N. Y., March 29, 1907, five individuals of *Amblystoma jeffersonianum*. Upon returning from his collecting trip he put them into a receptacle and immediately the smallest one (♂) embraced another (♀) exactly after the manner of the spotted newt. At Ithaca, April 2, after he had transferred them to a larger aquarium jar the same individual repeated the performance. The embrace was continued for sometime. Neither eggs nor spermatophores were subsequently laid, yet the fact is significant.

Of the intervals that exist between the first spermatophores deposited and the first eggs laid Professor Andrews[3] has noted that "24 hours or so" may intervene. Upon this point the following data may be of interest: in 1903, one interval of 2 days was recorded; in 1904, one of 4 days; in 1906, one of 6 days ;in 1907, 4 days in one pond, 5 in another, and 7 in a third.

Egg-laying generally begins about the first of April. In two or three of the last eight years eggs have been noted before that date. In this period the earliest record is March 20, 1903. In 1901 they did not begin depositing eggs until after the middle of April. The egg-laying for the species may extend over a month or more. Rarely do we find fresh eggs after May 1. In 1907, our latest record for fresh eggs is April 30; our latest record for fresh spermatophores in the same pond, April 27.

[1] Clarke, S. F., "Development of *Amblystoma punctatum*, Studies from Biol. Lab. of Johns Hopkins Univ., No. II., 1880, p. 106.

[2] Smith, B. G., "The Breeding Habits of *Amblystoma punctatum* Linn.," *American Naturalist*, XLI., No. 486, June, 1907, p. 388.

[3] Andrews, E. A., "Breeding Habits of the Spotted Salamander (*Amblystoma punctatum*)," *American Naturalist*, Vol. 31, p. 636.

The first egg records for the last eight years follow:

1900, April	6	
1901, April	13	
1902, March	28,	3 days after first appearance of the species.
1903, March	20,	7 " " " " " " "
1904, April	5,	4 " " " " " " "
1905, April,	11	
1906, April	14,	17 " " " " " " "
1907, March	30,	6 " " " " " " "

So far as I am able to determine there are very few observations upon egg-laying in nature. In 1878, Samuel F. Clarke made the following observations upon some captive females: "I was interested to find, after carefully watching the process a number of times, that the number of eggs deposited at a time depends upon accident. If the creature is disturbed, as by another individual striking against or touching it, or by the moving or jarring of the dish, she immediately suspends operations, and seeks some more quiet spot for the continuance of her labors. I have seen a single egg deposited and again a bunch containing one hundred and fifty. While the eggs are being extruded the animal usually lies with its anterior limbs extended laterally, while the hind limbs are curved around the opening of the cloaca and appear to assist in holding together the eggs as they are laid."

Egg-laying apparently takes place almost entirely at night. A chance discovery made while studying the early breeding habits of our local *Anura* may tend to confirm this view. At 9 P. M. of March 30, 1907, I found the *Amblystomas* of one pond swimming restlessly about its edges in considerable numbers and suspected that egg-laying was about to begin. At 9:30 P. M. another pond was visited and by means of an electric flashlight three different females of *Amblystoma punctatum* were found in the egg-laying position.

This pond is 30 × 15 feet in diameter, 2–3 feet deep, and the banks steep. The bottom is covered with dead leaves. In the pond are brush and growing smartweed (*Polygonum Hydropiper*). About the pond the first adult *Amblystomas* were taken March 24; the first spermatophores were deposited in the pond March 25; and the first eggs laid March 30.

All three females were laying at one time, and two of t three were simultaneously depositing upon two closely appos stems of smartweed, the vent of the lower one being about inch below that of the other. One female held on with bc hind- and fore-limbs. Her head was appressed to the side the stem. The second female was facing the first, her vent bei slightly lower. She grasped the stem only by her hind lim was semi-erect and inclined diagonally to the side. The ste were so small and close together, that had the second desirec she could not have clasped the stem with her fore-limbs wi out embracing the first female. In both the tail extended diag nally downward, no prehensile tendencies being noted in any the three.

The first female after about a minute disengaged herself a swam off. The second after a short time, did the same but v captured. Both bunches of eggs were at this moment no m than one half of an inch in diameter. In less than an hour th were two inches in diameter. The third female, only a foot aw had not been disturbed by the sweeping of the net. She h on by her hind limbs only, leaving most of her body free. T bunch of eggs was only an inch from the top of the stem. T female was not perfectly erect but slightly arched.

When the second female was killed she emitted many eg Evidently, the first bunch, normal in number, was not all s had to lay. Nor is it unusual to record *Amblystoma* eggs laid small bunches. In one of our temporary woodland pools, Mar 30, 1907, I found a stem of common nightshade (*Solanum D camara*) which had within a length of one and a half feet bunches of eggs, 15–20 eggs to a bunch. It is very doubtful each bunch represents a different female, when it is well kno that a female may have 150 or more eggs to deposit. It is mc natural to conclude that these bunches represent the egg co plement of one. The oviposition might have been interrupt the female might have crept along the stem or after a period emission she might have risen to the surface for air and th returned to the stem again as the swamp cricket frog (*Chorophi triseriatus*) regularly does.

In captivity females when depositing may have quite lo

intervals between the emissions of eggs. This spring a female taken at the height of the breeding season voided enough eggs to cover the bottom of a small aquarium jar. She was brought into the laboratory without an attendant male and after several hours began to deposit eggs. The deposition lasted ten days and the eggs first laid hatched. Captive females have more of a tendency to lay single eggs than in nature, yet single eggs have been recorded afield. Even before the female reaches the marshes or ponds she occasionally lays eggs. In several instances near the breeding grounds we have found under moist stones or logs one or two eggs laid by *Amblystomas*.

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A NOTE ON THE CHEMOTAXIS OF OXYTRICHA ÆRUGINOSA.

GEORGE WAGNER.

Oxytricha æruginosa, Wrzeniowski, is one of the *Hypotric*[illegible]*icha* occurring rather irregularly in laboratory cultures. Cultures [illegible]s of it, so far as my experience goes, are quite characteristic in t[illegible] the fact that they are distinctly brown in color, and decidedly m[illegible]ore alkaline than the average cultures of *Paramecium*.

Jennings and Moore[1] found that this organism differed fr[illegible]om the congeneric *Oxytricha fallax* by forming spontaneously den[illegible]nse aggregations like those of *Paramecium*. On the other hand th[illegible]ey found that unlike *Paramecium* it was not positive to carbon dio[illegible]x-ide or indeed to any acid. They found that if both forms w[illegible]re mounted on the same slide according to Jennings's now we[illegible]ll-known method, they would form separate aggregations, and th[illegible]at the members of each species would pass freely through collectio[illegible]ns of the other without being in any way detained. It is obvio[illegible]us therefore that the cause of aggregation in the two forms must [illegible]be different.

Several years ago I made an attempt, at the suggestion [illegible]of Professor Jennings, to learn somewhat more about this differenc[illegible]e. Unfortunately I was unable at that time to complete the wor[illegible]k, and I have not since been able to secure the necessary cultur[illegible]es of *Oxytricha*.[2] It seems wise therefore to publish the resul[illegible]t so far obtained even though they may seem fragmentary.

The spontaneous aggregations of *Oxytricha* on slides or eve[illegible] in small open dishes are remarkable in being much more den[illegible] than those of *Paramecium*, in being much more sharply circun[illegible] scribed, and in expanding much less rapidly. In certain mixe[illegible] mounts of *Paramecium* and *Oxytricha* I found that, probably b[illegible] mere coincidence, both forms congregated in the same area. I[illegible] such cases at first the area inhabited by both was the same, ex[illegible]

[1] *American Journal of Physiology*, Vol. 6, 1902, pp. 242–243.

[2] For the rest of this paper *Oxytricha* is to be considered as meaning *O. æruginosa*[illegible]

cept that *Paramecium* swam further out, possibly due to the greater momentum caused by their larger body and faster rate of motion. But soon *Oxytricha* began to shun the center of the drop, repelled by the carbon dioxide exuded by the *Paramecia*. But they were held by their own attractive excretion in a ring around the outside of the area. Within thirty minutes however the *Oxytricha* had moved so far outward that the *Paramecia* were entirely separated from them; shortly afterward the aggregation of *Oxytricha*, due undoubtedly to its attenuated character, broke up, while the *Paramecia* were still densely crowded together. In a second similar group, where however the relative number of *Paramecia* was much smaller, the two organisms remained evenly distributed throughout the drop, and the area was extended at the same rate for both of them. Why this difference? Very likely because the smaller number of *Paramecia* acted less rapidly in making the center of the drop uninhabitable for *Oxytricha*, while the *Oxytricha* in moving outward, by their own excretion of carbon dioxide kept the whole area acceptable to *Paramecium*.

Paramecia in culture water, after aëration, react positively to distilled water or weak acid. When in distilled water they react positively to weak acid but negatively to stronger acid or to alkali. *Paramecium* therefore usually seems to seek an optimum region, weakly acid in reaction. (Of certain exceptions to this I hope to speak in another paper.) The relation of this to the general life activities of *Paramecium* has often been pointed out by Jennings.

A culture of *Oxytricha*, aërated in similar fashion, reacts quite differently. If such an aërated culture is placed under a cover glass on a slide, and a drop of distilled water is introduced, the center of this drop remains free from *Oxytricha*. Such as swim into the edge of the drop give the typical motor reaction, long before they reach the center, and then swim outward, but only for a short distance; for there exists now also an outer boundary bringing forth the same reaction. The result is that there is soon formed a ring-like aggregation, quite like that of *Paramecium* in presence of a drop of stronger acid. If such an aggregation is left undisturbed for some time, the *Oxytricha* usually soon begin to collect in some one portion of the ring, and within five or six minutes form there a very dense aggregation.

When similarly aërated cultures are tested with alkaline solutions it is found that with very weak alkalies, such as *n*/1,000 — *n*/1,500 KOH, the results were quite parallel with those with distilled water. That is, there was a ring-like aggregation around the edge of the drop. When the culture was made slightly acid, and then a drop of hydrant water (alkaline) was introduced, there was usually a more or less distinct ring formation, occasionally a complete negative reaction. The hydrant water used was decidedly more alkaline than the ordinary cultures of *Oxytricha*. That *Oxytricha* forms a ring around such a drop, might make one suppose that here we find an optimum of slight alkalinity for *Oxytricha*. But such proves not to be the case, at least not in so simple a form. Many experiments showed that so far as my cultures went, *Oxytricha* was always negative to stronger KOH (*n*/200) often with formation of a ring, and without exception wholly indifferent to weaker KOH. There was never a gathering within the drop no matter what dilution was used.

It seems then that there is really no optimum strength of alkali in which *Oxytricha* will gather. In those cases, just mentioned, where there is a ring formed, we must rather suppose that the alkali plays no part except to repel, but that the water acts as diluent to some other substance present in the culture, attractive to *Oxytricha*, but present in super-optimum amount.

In cultures made slightly acid, *Oxytricha* reacts to weak alkali either by collecting in it (rarely), or by forming a ring around it, depending on the strength of the alkali. Here again it is probably not an optimum condition somewhere in the alkali that causes the result, but rather a reduction to an optimum amount of some other substance present in the culture medium, coupled with a strongly negative reaction toward the acid. For if instead of a weak alkali we use a drop of culture liquid, which is normally weakly alkaline, we find an immediate and strong positive reaction in all cases. It is then not alkalinity but an unknown factor that produces this result. That alkalinity is not the factor is shown by further experiment.

In one culture it was found that *Oxytricha* when exposed in culture water to a drop of distilled water reacted purely negatively, and this whether the culture was aërated or not, or

whether the distilled water was aërated or not. Many repetitions always gave the same result. It seemed possible that here was a culture with optimum alkalinity. If so an increase in its alkalinity ought to produce ring formation around a drop of distilled water. To test this the culture was made rather strongly alkaline by addition of $n/100$ KOH, and to a mount a drop of distilled water was added. But the result was as before. In every case this one race, even when the culture was made many times more alkaline than in its natural state there was still a purely negative reaction toward distilled water. There was evidently an optimum condition in this culture, but it was not an optimum of alkali.

Some other cultures varied from this by showing only an incipient tendency toward ring formation with distilled water, in others again *Oxytricha* soon approached very close to the center of the drop. It seems obvious therefore that various cultures differ widely as to their approach to an optimum state, but that this variation is not, primarily at least, correlated with alkalinity. It seems plain to me, that the reactions of cultures to distilled water are due not to alkalinity but to the effect of some other yet unknown condition. Distilled water represents to *Oxytricha* not merely a transition stage between weak alkali and weak acid. If it did one might expect *Oxytricha* (always, when in culture solution, negative to even very weak acid) when placed in weak acid, to react positively to distilled water. A culture was made acid and aërated, then a drop of distilled water introduced on a mount. In some cases there was indifference, in a few there *was* a positive reaction, but in by far the greatest number of cases the organisms gave a negative reaction toward the distilled water, as most alkaline cultures would have done, sometimes with ring formation.

The same acidified culture however reacted positively to a drop of its own unacidified culture water.

It seemed obviously desirable to find out something of this unknown constituent leading to these results. Most of the experiments seemed to show that such a substance was present usually in more than optimum amount, hence the frequent occurrence of ring formations around diluents. It should be noticed

that in spite of our knowledge of the lack of reaction of *Oxytricha* to carbon dioxide, I took the precaution of aërating the cultures in all the above experiments, except where otherwise stated. The reason for this precaution lies in the following results.

A portion of an *Oxytricha* culture was taken and thoroughly aërated by squirting it repeatedly into a watch glass from a pipette. A mount was immediately made and a drop of non-aërated liquid from the same culture introduced. The result was a strong positive reaction toward this non-aërated drop. To an entirely similar mount a drop of *aërated* culture water was added. The organisms were entirely indifferent, swimming in and out of the drop, with no sign of reaction. Thirdly, a non-aërated mount was now made, and a drop of aërated culture water was added. The drop remained free from *Oxytricha*, which reacted negatively at its outer edge. If to a non-aërated mount a non-aërated drop was added, there was again indifference. These experiments were repeated many times, always with the same results. It is evident therefore that a non-aërated culture contains some substance toward which *Oxytricha* reacts positively, and that this substance can be removed by aëration. It must therefore be either volatile, gaseous or easily oxidized.

But an *aërated* culture will also react negatively to hydrant water or to distilled water, often with ring formation. That such ring formation occurs with distilled water shows that what happens here is due to another, non-volatile substance present in the culture, toward which *Oxytricha* also reacts positively at proper concentration. This second substance also seems to be present usually in super-optimum amount. This second substance may sometimes be the alkali. That it is not always such is of course well shown by the case mentioned above, where a culture, after addition of much KOH and of aëration, still remained purely negative to distilled water. This experiment also shows that it is not merely a matter of osmotic pressure.

An interesting experiment in this connection is that in which *Oxytricha*, previously gathered into hydrant water by electrotaxis, were mounted and a drop of slightly acidulated culture water added. There was quickly formed a dense ring around the drop, which was long maintained. It was evident that here

one or both the unknown substances attracted the organism, while the acid above a certain strength repelled it. The *Oxytricha* under the influence of these opposite forces, was held along a line where the two balanced each other. According to Jennings, an exactly similar phenomenon occurs in *Paramecium*, when exposed to a mixed solution of acid and table salt.

It appears then that we have here at least two unknown substances, one volatile, the other not, affecting the chemotactic reactions of *Oxytricha*. The presence of two such substances, whatever may be their origin, helps to explain away a difficulty which I think has been overlooked. In the ring formation so commonly occurring, the *Oxytricha* are at first distributed equally around the circle. They are held within it, so we suppose, because some substance is there in optimum quantity. As the drop, say of distilled water, diffuses this optimum area moves outward, and the organisms move with it. However, if undisturbed, the *Oxytricha* will soon be congregating in some one portion of the circle and forming a dense mass, entirely similar to the ordinary spontaneous aggregations. Now on the supposition that there is only one attractive substance concerned, such aggregation in one part of a circle which is supposed to uniformly contain an optimum amount of this attractive substance is hard to understand. But if we suppose that in the dilution of one of two attractive substances to an optimum strength the second substance is reduced below its optimum, then it is easily seen that the meeting of two or three *Oxytricha* in some part of the circle would soon tend to bring this second substance to its optimum strength in that part of the circle. Then naturally any further individuals entering this smaller area would be retained there, in similar manner to that by which they are retained in the circle as a whole.

I can only add that there is some evidence that these substances are formed with the development of a colony, and that the character of the chemotactic reaction itself is altered in the course of such development. A culture of *Oxytricha* rejuvenated by addition of fresh hay and water, acted very anomalously. The creatures were positive to distilled water, as well as to $n/100$ HCl. But two days later all this had changed, and the reactions to both substances were negative.

In older colonies the attractive substances seem to develop far above the optimum amount. In such culture water the amount can be again reduced by filtering and allowing the filtrate to stand over night in an open dish. Whether such reduction is by volatilization or oxidation, or by both, I do not know. Certain it is that to such a liquid *Oxytricha* taken from the same culture from which it came, react positively.

Ordinarily a mount from any culture will be indifferent toward its own filtered culture water, if both remain unaërated. But if from a culture a highly concentrated mass of individuals are taken and kept for a few hours in a small watch glass, and then mounted, they will give a strong positive reaction toward a drop of water from their own original culture. Evidently here a close aggregation of individuals has supercharged the water, hence a positive reaction to the original culture solution of lesser strength.

Peculiarly, of two colonies of *Oxytricha* each, unaërated, was negative to the culture water of the other, but when aërated it became positive, the introduced drop in both cases being unaërated. This again points to a marked difference between cultures, as several other facts do. To this phase of the subject I expect to recur in another paper.

In a search for a clue to the unknown attractive substances, I tried many organic acids, sugar, urea, acetic aldehyd, potassium cyanide, ammonia, and a few others, but always with purely negative reactions. The possibilities of the case are of course nearly endless, but should opportunity offer, I would wish to experiment with organic sulphur compounds, and with compounds of ammonia, and this purely on the empiric basis of the odors of *Oxytricha* cultures.

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of them arrange themselves in the form of a ring, inside of which are the other five, which in this division are quite separate.

The chromosomes seem to vary considerably in size and shape, but this is merely due I think to various degrees of foreshortening, produced by slight differences of position. However, some are

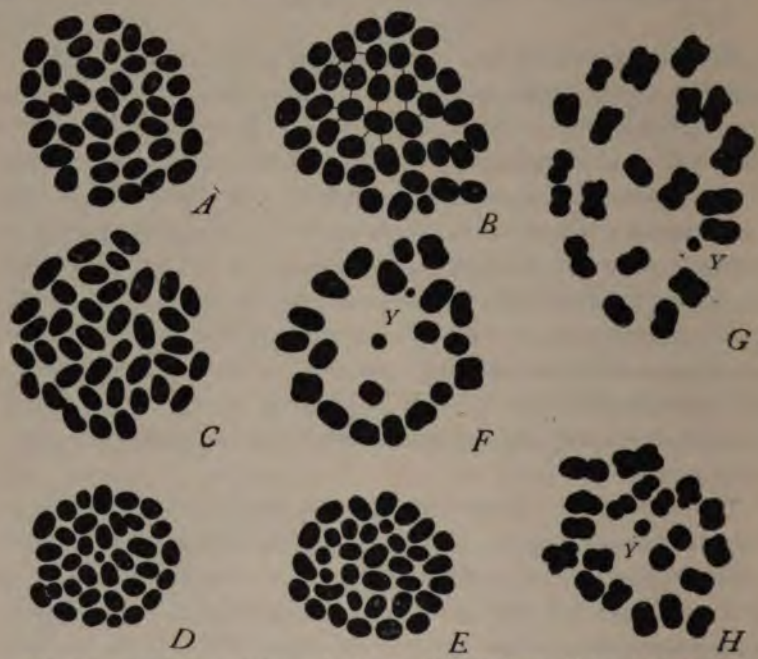

FIG. 1.[1] *Galgulus oculatus.* *A*, *B* and *C*, metaphase of female (oögonia or follicle) cells, polar view, showing thirty-eight chromosomes; *D* and *E*, metaphase of spermatogonial cells, polar view, showing thirty-five chromosomes; *F*, *G* and *H*, metaphase of the first spermatocyte division, polar view, showing twenty chromosomes and the granules γ. *A*, *B*, *C*, *D* and *E* are magnified 3,105 diameters and *F*, *G* and *H* 2,009 diameters.

evidently quadripartite and some bipartite. From my figures some may be inclined to believe that the granules may be chromosomes, but the number twenty is verified by the fact that the

[1] All of the figures were very carefully drawn with a camera, a 2 mm. oil immersion (Spencer) and compensation ocular 12 (Zeiss). Some were enlarged once and some twice by means of a drawing camera which magnified $1\frac{6}{10}$ diameters. Although some error is unavoidable at such an enlargement, I have used great care in correcting, and the chromosomes are not schematized in the least, as can be seen by a comparison of the drawings, Fig. 2, *B* and *F* with the photographs, Fig. 3, *A* and *D*.

metaphase figures of the second division, without exception show twenty chromosomes. Again I have counted the chromosomes in serial sections of the prophases before the nuclear membrane breaks down. At this time, there are no granules within the nucleus and several counts showed twenty chromosomes each.

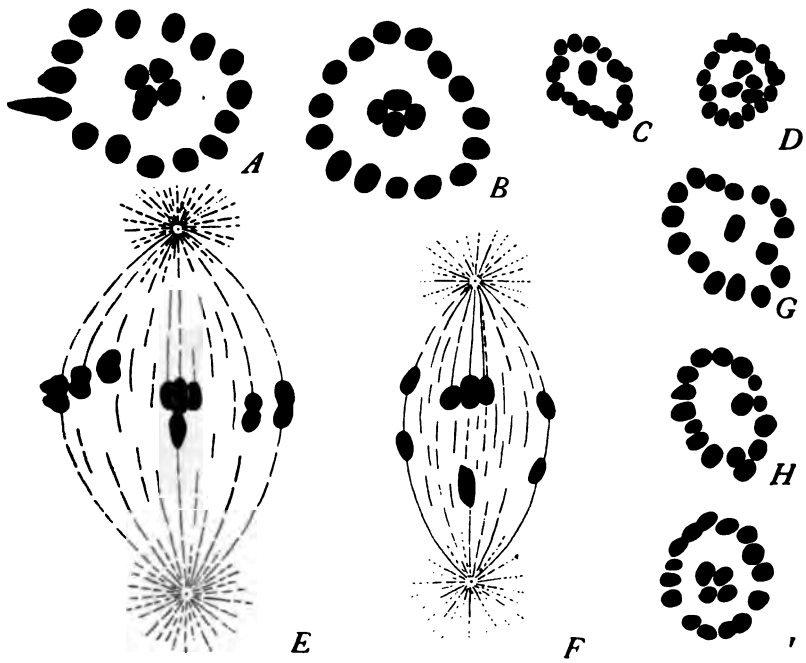

FIG. 2. *Galgulus oculatus.* *A* and *B*, metaphase figures of the second spermatocyte division, polar view, showing the ring of fifteen chromosomes and the pentad group in the center — in *B*, the chromosome beneath the four group could not be shown without displacing it; *C* and *D*, late anaphases of the second division, polar view, showing the unequal distribution of the chromosomes; *E*, side view of metaphase, second division, showing the typical arrangement and position of the pentad group — the spindle in both *E* and *F* is diagrammatic and merely shows size relations; *F*, side view of the early anaphase, second division, showing the manner in which the chromosomes of the pentad group separate; *G*, *H* and *I*, early anaphases of the second division, showing the chromosome distribution to the two classes of spermatozoa. All figures are drawn on the same scale and magnified 2,009 diameters.

The second division, which follows immediately after the first, shows a remarkable regrouping of certain of the chromosomes. Fifteen of the twenty take up the position of a ring, within which is a definite compound element formed by the remaining five.

These are now arranged in a pentad group, which always shows the same composition and occupies the same position. Four of these five chromosomes are grouped very closely together and lie in one plane, while the other one is either above or below this group of four, lying close to them on the other side of the equatorial plane (Fig. 2, *A*, polar view of the equatorial plate; Fig. 2, *E*, and Fig. 3, *C*, side views). The fifteen chromosomes in the ring divide equally, while the chromosomes of the central pentad do not divide individually, but the group as a whole separates in such a manner that one chromosome passes to one pole

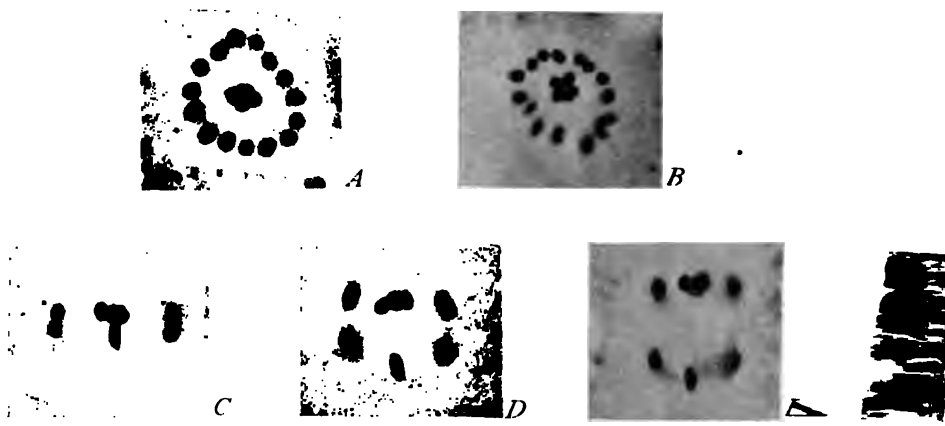

FIG. 3. *Galgulus oculatus*. *A*, metaphase figure of the second division, polar view, showing the ring of fifteen and the four chromosomes of the pentad group near the middle — the fifth chromosome of the pentad group could not be shown, as it lies beneath the four; *B*, early anaphase of the second division, polar view, showing the nineteen chromosomes which go to one pole; *C*, metaphase of the second division, side view, showing the typical position and arrangement of the chromosomes of the pentad group; *D* and *E*, anaphases of the second division, side view, showing the manner of separation of the pentad group, four chromosomes of which go to one pole and one to the other — only three chromosomes of the four group show, as all of them do not lie in the same plane. The photographic enlargement is 1,500 diameters.

and the other four to the other pole (Fig. 2, *F*, and Fig. 3, *D* and *E*). Two classes of spermatozoa are thus formed, which contain sixteen and nineteen chromosomes respectively. The early anaphase illustrating these two classes is shown in Fig. 2, *G*, *H* and *I*, and Fig. 3, *B*; the later anaphase in Fig. 2, and *D*.

It yet remains to bring these two classes of spermatozoa into relation with the spermatogonial and oögonial numbers. Unfortunately but two spermatogonial metaphase figures are shown with entire clearness. They agree in showing each thirty-five chromosomes (Fig. 1, *D* and *E*). It may be thought that this evidence is not sufficient to establish the number with certainty. However, the number in these two cases is quite unmistakable, and as will be shown, it is the number to be expected from the numerical relations observed in the spermatocyte chromosomes and in the female. The female number (oögonia or follicle cells) is, without a doubt, thirty-eight (Fig. 1, *A*, *B* and *C*). The analogy which exists between the numerical relations here and in those forms with an odd chromosome, where the female number is one more than the male, makes the evidence all the more convincing that thirty-five is the male number in *Galgulus*. It is evident from these facts that the reduced female group must contain nineteen chromosomes; and that accordingly females are produced upon fertilization by the nineteen-chromosome class of spermatozoa; males upon fertilization by the sixteen-chromosome class.

Egg 19 + spermatozoön 16 = 35 (♂)
Egg 19 + spermatozoön 19 = 38 (♀)

So far, I have not ventured a new name for these characteristic chromosomes which make up the pentad group of the second division. At present I shall simply refer to them as differential chromosomes as Wilson, '06, has done in the case of the idiochromosomes.

Between the spermatogonial and the first spermatocyte divisions occurs a very prolonged growth period, during which the cell diameter increases approximately five times. Throughout this growth period, between synapsis and the formation of the chromosomes preparatory to the first maturation division, persists a large deeply staining body, more or less comparable in time of appearance and behavior, to the chromosome nucleolus of those forms in which it represents the odd chromosome or the idiochromosomes. I have not fully followed the history of this

structure in *Galgulus* and hence cannot definitely say what it is. It is possible that it is formed by the fusion of the five chromosomes which later appear in the pentad group of the second division.

At present there are not sufficient data to show the exact relation between this type of chromosome distribution and those already described. For this reason, I shall not attempt to homologize them in detail. Nevertheless, there is an evident similarity between the behavior of the pentad group as a whole and that of a single pair of idiochromosomes. In a general way, the four chromosomes of this group which go to one pole have the same relation to sex-production as a single large idiochromosome, while the one chromosome which goes to the other pole may be compared to a small idiochromosome.

With the discovery of this new type of differential chromosomes, it becomes more evident that the sexual differences of the chromosomes, even in the same order of animals, by no means conforms to a single numerical rule. In forms with one pair of idiochromosomes, the two sexes have the same number of chromosomes; in those with the odd chromosome, the female has one more than the male; and in *Galgulus*, the female has three more chromosomes than the male.

In many forms, as has been shown by Montgomery, Sutton and others, the spermatogonial and oögonial chromosomes may be paired two by two in respect to their size relations. In *Galgulus*, the chromosomes are too nearly equal in size to be readily paired, and the differential chromosomes are indistinguishable from the others.

Since in *Galgulus* there are two classes of spermatozoa; since the spermatogonial number is thirty-five and the oögonial thirty-eight, we have another support to add to the view that the two classes of spermatozoa are respectively male and female producing. In a recent paper, Correns has demonstrated by experiment, that in some plants there are two kinds of male germ cells produced in equal numbers, and that these two kinds are male and female determinants.

ZOÖLOGICAL LABORATORY,
COLUMBIA UNIVERSITY,
February 21, 1908.

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OCCURRENCE OF THE FRESH-WATER MEDUSA LIMNOCODIUM, IN THE UNITED STATES.[1]

CHAS. W. HARGITT.

In a preliminary note to *Science*, which appeared in the issu of November 8 (1907), attention was directed to the occurrenc of *Limnocodium* in this country, and some brief account given o this circumstances and conditions of its appearance. As indicatec in that report a few living specimens came into the possession o the writer through the courtesy of the United States Bureau o Fisheries, and were kept alive and under observation for a perioc of some ten days. Since it was confidently anticipated that a further supply of specimens would be received very soon nc particular care was taken to prolong the life of the first supply A few were used for some simple experiments, to be described ir a later section, and some preserved for histological purposes It is with extreme regret that I have to record the utter failure o attempts to secure a further supply of living specimens, though from no particular blame of those concerned. At least one lot was forwarded to the writer at Syracuse, and left Washington in good shape, but from some cause not clear all were dead when received, having perished in transit. Other supplies were immediately sought, but within a few days the very disappointing advice was received from the director of the aquarium that the medusæ had suddenly disappeared. Quoting his own words: "When I went to the tank where they have been so plentiful for the past eight weeks there was not one to be seen, and apparently they have disappeared as mysteriously as they came. Possibly on account of the cold weather they may have gone to the bottom of the tank, among the pipes where I cannot get them, but if I discover them again I will advise you at once." This letter bore date of September 27. In a later letter, December 3, I was advised that no specimens had appeared during the interval of two months, and from the history of these medusæ in Europe this is only what might have been anticipated. Therefore, with the first chapter

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

of the present case thus closed, the details, so far as known, may properly be formulated and made available.

Locality.

The medusæ were discovered in the aquarium of Mr. W. B. Shaw, a florist, who for many years has cultivated various aquatic plants, among them several species of tropical, or subtropical water lilies, *Nymphæa zanzibarensis*, *Cabomba* (*Caroliniana?*), and a species of *Ludwigia*. But, *all these were reared from seeds*, no grown plants having been at any time imported or introduced into the tanks. And at no time has the *Victoria regia* been grown here, a fact of no small interest in relation to earlier suggestions as to the problem of the transportation of the medusæ from tropical waters.

The hot-house contained in all six tanks, each three feet wide, three feet deep, and about twelve feet long. These were all stocked in the same way, and with about the same sorts of plants, and a species of Paradise fish. The aquaria are of the balanced sort, and have been in use for some six or eight years without material change of water or organisms. The aquaria are used chiefly during the winter by Mr. Shaw including late fall and spring, for the purpose of carrying over certain of the more delicate tropical plants from season to season, and stand idle during summer, water being added to replace the loss due to evaporation.

Of these six tanks, alike in construction, water supply, and other features, medusæ appeared in *one only*, and that about midsummer, flourishing during the hot weather and promptly disappearing with the approach of autumn cold. When first discovered they were in considerable numbers and of various sizes "some as small as a pin's head and some one fourth inch in diameter" The largest specimen which came to my hands measured about 9 mm. in diameter by 4 mm. in height of bell.

Hoping to obtain some clue as to the hydroid stage I obtained through the courtesy of the Bureau of Fisheries a collection of various plants growing in the tanks with scrapings of algæ, slime and other debris from the sides and bottom of the tank. This was promptly and carefully examined for hydroids, and frequently

during succeeding days, but no trace of a hydroid was found. The material was kept in my laboratory, which has a fairly constant temperature, for several weeks, indeed months, and repeated examinations made from time to time, but no trace of any hydroid form appeared, though species of Protozoa and rotifers continued to live and thrive quite normally. Such are the facts as to locality and conditions under which the medusæ were found. Their similarity to those concerned in the appearance of the same medusa in London many years ago is quite striking. Its occurrence later at several places on the continent has been recorded by several observers. The most recent is that of its appearance at the botanical garden at Munich in the basin containing the giant water lily, *Victoria regia*. Boecker's account, which appeared in the *Biologisches Centralblatt* (Bd. XXV., p. 605, 1905), agrees in most respects with the earlier records, and with the foregoing as well. He regards the Munich species as probably identical with *Limnocodium sowerbii*, the occurrence of which in the Victoria tanks of Regents Park, London, had been variously described by Lankester (*Quar. Jour. Mic. Sci.*, Vol. XX., 1880), Allman (*Jour. Linn. Soc.*, Vol. XV., 1881), and Gunther (*Quar. Jour. Mic. Sci.*, Vol. XXXV.). Some further notice will be taken of Boecker's records in a later connection.

General Features and Relations.

The Washington species agree in most respects quite closely with the original descriptions and figures of *Limnocodium sowerbii* of Allman and Lankester. As compared with the verbal description of Boecker (*op. cit.*) there are some slight differences. For example, he says that the Munich species are characterized by a rather high-arched bell. In my species the bell is rather low, often disc-like. Figs. 1, 3, 4, drawn from life in different aspects, will make this point more evident and striking. However, this difference I do not consider of very great importance

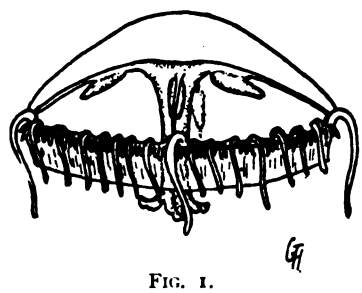

Fig. 1.

ral things might conspire to emphasize or exaggerate it. As ell known, age has much to do with the shape of hydrome-

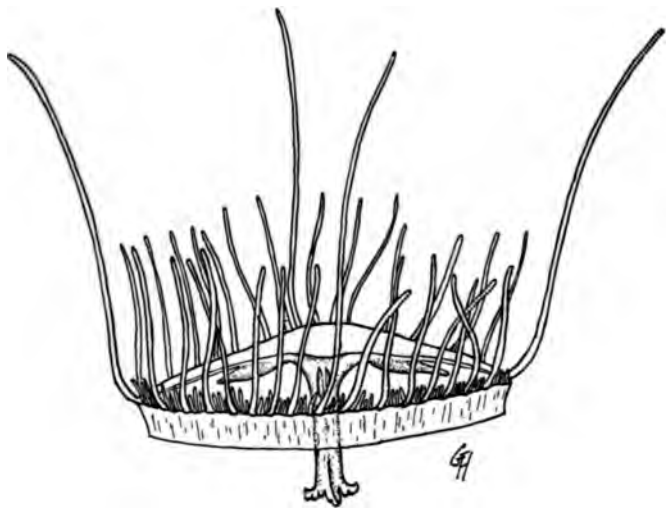

FIG. 2. Medusa, floating aspect.

e, young specimens often being hemispherical, while at matu- they may become quite disc-like. Again, there are often ced individual differences which have to be considered in nating the mean shape of the species. Further, preserved imens are almost always more or less contracted, and hence seem to be much more highly arched than is the case in life. should be observed in this connection that the specimens vn in the figures present very different aspects. Fig. 1 repre- s the medusa in an average swimming attitude, the tentacles e or less contracted, the manubrium somewhat contracted, the bell rather higher than shown in Fig. 2, which represents creature in the floating attitude, body and organs generally

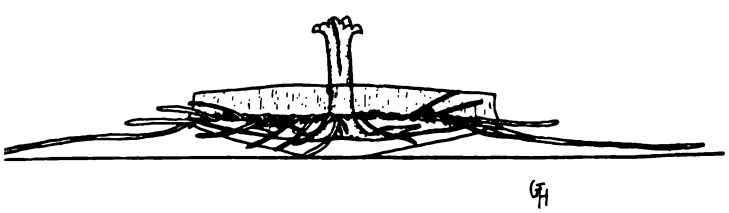

FIG. 3. Medusa, resting normally on bottom of aquarium.

extended, and more or less passive. In Fig. 3 the specimen is represented in a rather characteristic state of repose often observed, resting on the exumbrellar surface on the bottom of the aquarium. Here again, the creature is in a condition of general relaxation, the manubrium extended and in position to take such prey as may come within its reach. The tentacles also are much extended, a condition quite common when in this pose.

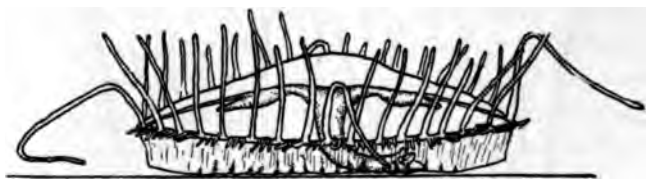

FIG. 4.

In Fig. 4 is shown a less familiar attitude of the creature. Though not especially rare, the position seems to indicate a condition of fatigue, though the tentacles are more or less erect. A comparison of the several figures, representing as closely as possible the living aspects of the creature, will show the general relation of tentacles, velum, manubrium, shape of bell, etc., to the state of activity or repose, as the case might be.

TENTACLES.

There are four perradial tentacles, conspicuous as to size and length, as shown in the several figures. In certain cases it was possible to distinguish apparently, a series of four interradial tentacles though they were not conspicuously differentiated as to size or length. The tentacles are quite numerous, especially in older specimens, and arranged in several series, younger ones arising from the lower, or proximal portion of the margin. While the aspect exhibited by these organs in the figures might seem to indicate more or less rigidity of structure, this did not appear to be the case in the living specimens. They were quite flexible and highly contractile.

In describing the tentacles of the Munich specimens Boecker (*op. cit.*) was unable to confirm the account given by Günther as

to the insertion of the base into the gelatinous margin of the bell, believing them to be quite free. He does not state whether he had studied this feature in sections, or from simply observing the living specimens.

On this point my specimens show considerable differences. In the living condition, the larger tentacles, while capable of great freedom of movement, are yet inserted in notch-like depressions in the margin, as shown in Fig. 5. As studied in sections they

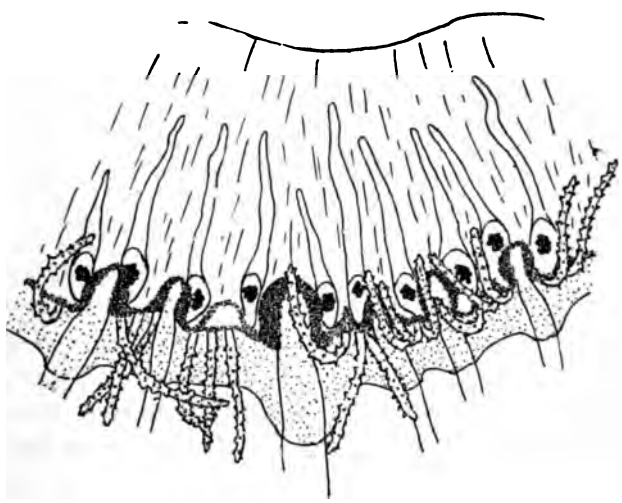

FIG. 5. Portion of margin enlarged, showing tentacles, velar canals and marginal bodies.

show something of the condition figured by Günther, though very much less marked, the degree of adnation being much less than in his description. Furthermore, I should not consider the degree of fusion as having any very evident relation to the aspects of the tentacles exhibited by the living medusæ. On the contrary, the varying aspects of these organs under differing conditions of activity or repose are such as to strongly suggest a very different conclusion and interpretation.

A further tentacular feature remains to be considered, namely, that of structure. Both Allman and Lankester described the tentacles as solid, though differing more or less from that found in other medusæ with solid tentacles. Günther has later shown that these conclusions were erroneous, and attributes

the error as possibly due to an examination of very young tentacles, or to tentacles in a state of high contraction.

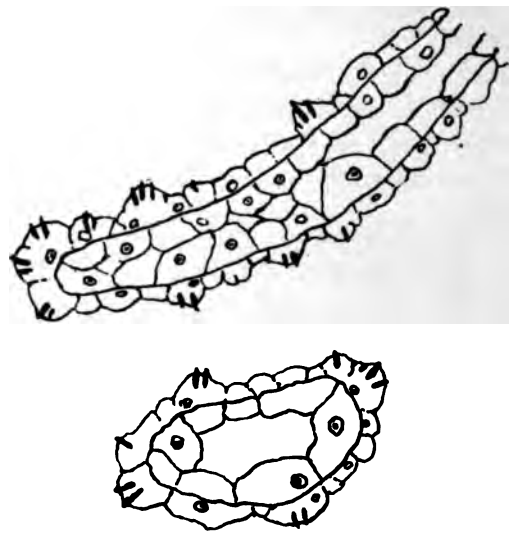

FIG. 6.

My own sections confirm the results of Günther. In sections of very small tentacles it is quite difficult to distinguish the lumen of the tentacular axis. But in larger specimens this is quite easily demonstrated, and it seems rather strange that two such capable observers should have failed to recognize this feature. Fig. 6 shows camera sketches of both cross, and long sections of tentacles, and Fig. 7 is a photograph of the terminal portion of a tentacle made under the microscope.

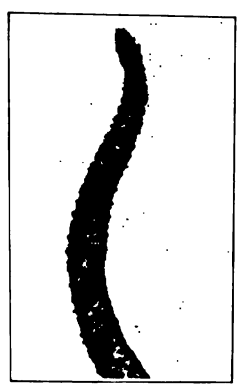

FIG. 7. Tentacle of Limnocodium.

In this connection I desire also to point out that in none of my specimens have I found the very marked papillated aspect of the tentacles as represented by both Lankester and Günther (*op. cit.*), and later by Browne (*Quar. Jour. Mic. Sci.*, Vol. 50, Fig. 3, pl. 37). These papillæ are specialized organs bearing nemato-

cysts, according to these observers. But I have failed to find any well defined cases of such highly specialized nematophoric structures. The figures, both from the drawings and the photograph alike, confirm this statement. I cannot resist the impression that the figures of the former must have been drawn from greatly distorted specimens due in part perhaps to mode of preservation, or that they may have been made from very young tentacles in which the nematocysts are relatively more conspicuous. A comparison of various of my specimens fails to reveal any essential differences, even where various modes of preservation were employed. An examination of the specimen represented in Fig. 7 will show fairly well the general distribution of the nematocysts, and also the tendency to an annular disposition similar to that common to other hydromedusæ.

Marginal Bodies and Velar Canals.

Concerning these organs I have made no attempt to critically investigate anew their structure or development, beyond a more or less careful review of the work of Lankester, and the later work of Günther (*op. cit.*). So far as I have gone my observations confirm that of the previous investigators. Fig. 5 is a camera sketch of a portion of the margin and velum showing the general aspects of the several organs, tentacles, marginal bodies and capsules, and velar canals. But while confirming the facts described by Lankester as to the structure and relations of the marginal bodies, I have found no convincing evidence of his rather dogmatic assertion that "The refringent body is nothing more nor less than a modified tentacle." This may have sufficed its author at the time it was made, and was quite in harmony with then current views concerning these organs in general. But the time has long since passed when the mere matter of similarity of structures, real or imaginary, is any sufficient warrant for the vaulting conclusion involved in the above quotation. In another connection (*Jour. Exp. Zoöl.*, Vol. I., p. 86), I have taken occasion to question the long current assumption that in Scyphozoa the rhopalia are metamorphosed tentacles. Later observations have only served to deepen the conviction, and I believe adequate investigation will confirm the view that these organs are not, in

any strict morphogenic sense, directly derived from tentacles, particularly such bodies as those here under consideration.

Reproduction.

There is nothing to add to the former announcement as to this feature. Only male specimens have been found. These were in various stages of maturity, some quite young, others sexually mature, with gonads bursting with ripe spermatozoa. In the absence of additional facts there is no particular call for speculations as to the significance of this singular sexual phenomenon.

Affinities of the Medusæ.

Concerning the question of the specific or generic relations I find little occasion for difference of opinion or doubt. That our specimens belong to the genus *Limnocodium* seems thoroughly clear and certain. That it is also specifically identical with the European species, *L. sowerbii* Lankester, seems almost equally certain. I have pointed out certain features of difference, such as the shape of the bell as compared with Boecker's description, and peculiarities of tentacular structure, rather sharply different from those described by the earlier observers. Yet neither of these, nor both combined are such as to warrant the probability of a distinct species. Hence the suggestion made in my preliminary report may be here reiterated, namely, that the American species is almost certainly *Limnocodium sowerbii*.

The following characters may be regarded as fairly diagnostic of the species: Bell rather low, two or three times as broad as high, discoid in shape, especially when floating freely as shown in Fig. 2. Size from 5 to 9 mm. in diameter, by about 4 mm. in height, or less in average specimens; radial canals four, rather capacious, extending from stomach to marginal canal, which is triangular in shape and large; gonads four, suspended from the radial canals about midway between the stomach and margin, pouch-like in shape with the smaller or distal end free, as shown in the figures, pale greenish in color; manubrium long, and with four more or less crenulated lip-like lobes which are of greenish tint, and extending usually considerably beyond the velum; tentacles very numerous, the four perradial ones being conspicuously

play. Still others have contended that various species react very differently to a given stimulus, and that an element which may prove a stimulus in one case may prove inhibitory or even toxic in another case. It is quite certain, therefore, that the last word has not yet been said concerning this perplexing problem. And so far from the rather dogmatic declaration that "it is only the presence of Ca ions in the blood which prevents the muscles of our skeleton from beating rhythmically in our body" we may better assume an attitude of open skepticism, or at least suspend judgment long enough to perform a few more experiments! In the lack of material for such experimentation in the present case it occurred to me to try in a very simple fashion the effects of perfectly pure, that is distilled, water on the medusæ. In the absence of the ions of the aforementioned elements, Na, Ca, K, etc., in such water should we get any of the results usually attributed to them? The problem seemed simple enough, but not too simple to be unworthy of a trial. Accordingly the following experiments were made:

1. Specimens were transferred from the water in which they had been sent from the aquarium to that of the ordinary tap-water of the laboratory in order to see whether any appreciable effect would follow. But none could be observed; hence they were thereafter freely transferred to such water as circumstances seemed to suggest from day to day. Later tests of this water showed it to be so free from any of the salts in question as to be indistinguishable by the ordinary chemical tests.

2. A single specimen was next transferred to a jar of distilled water. It moved rhythmically for thirteen beats, then paused. Then followed six beats succeeded by another pause of longer extent during which it floated downward sinking to the bottom. Here there followed five beats and a pause of five minutes. At this time it was transferred back to normal water with the result of prompt resumption of rhythmic activity, but of a more rapid rate, nearly or quite twice that before the experiment was begun. This continued for nearly ten minutes, when the rhythm gradually returned to the normal rate.

3. Another specimen tested in the same way gave almost identically the same reactions.

4. The next specimen, somewhat larger than either of the former, was transferred to distilled water, and pulsed rhythmically twenty-three times, then paused as had the former. Then followed thirteen pulsations with another pause of similar extent, during which it gradually sank to the bottom where it remained motionless for three minutes. It then resumed its rhythmic swimming and continued without further pause for ten minutes when it was returned to the normal water.

5. After about twenty-five minutes all three specimens were transferred at once to a fresh jar of distilled water. In this case the reactions were similar throughout, though with certain individual differences such as one might expect. All swam rhythmically for a half minute, and then paused as in the previous cases. The rhythm was resumed and after a brief time two of the specimens paused again, and as before sank to the bottom of the jar, the third continued swimming. After a short rest those on the bottom resumed their swimming, and all continued for nearly half an hour, just as in normal water. They were left in that condition for twenty minutes longer, when two were found to have sunk to the bottom once more and seemed to show sign of discomfort, such as the contraction of the tentacles, drawing in of the margin of the bell, etc. The third while still swimming, was evidently showing unusual signs of fatigue, the movements being more or less feeble and uncertain. All were at once transferred to normal water. Two soon showed signs of recovery, the third however, continued in a state of collapse and failed to further recover. All had evidently been injured by the long period in the distilled water, and one fatally, since it was later found dead without indications of any recovery in the normal water.

Such in briefest outline are the facts resulting from the few experiments made. It should be stated that they were variously repeated, and with such degree of constancy in behavior as to suggest their perfectly trustworthy nature. Still other experiments of the same sort would have been made, but for the confident anticipation of a large supply of fresh specimens.

In reviewing the literature of this medusa it has been gratifying to find that some experimental work had long ago been done

upon it. In 1880 Romanes (*Nature*, June 24, also "Jelly fish, Star fish, etc.," 1885), whose experiments on various medusæ are well known classics, took occasion to repeat certain of them on *Limnocodium*, chiefly in relation to light, temperature, tactile localization, and reactions to sea water. In most respects he found the reactions of *Limnocodium* comparable in almost every respect with those performed on marine species. His experiments in transferring the specimens to sea water was to test their tolerance to a medium and environment to which their supposed ancestors must have long been familiar, are interestingly the very opposite of those above described. He had previously proved that marine species were unable to endure a transfer for more than ten minutes to fresh water, seldom for more than five, and then with evident signs of more or less severe injury. In the reverse tests with *Limnocodium* he found that no reaction was apparent for about fifteen seconds, when a more or less sudden collapse resulted, with tetanus-like spasms of increasing intensity till all sensory reaction ceases, and after a short time death ensues. An exposure to sea water for even one minute may end fatally, even if the creature be promptly returned to fresh water. Similar results followed an immersion in dilute sea water though with less promptness. In very reduced sea-water ($\frac{1}{10}$), spontaneous activity may continue for some time, but with gradual decline till but slight response is given to stimuli.

In conclusion he states "It will be seen from this account that the fresh-water medusa is even more intolerant of sea water than are the marine species of fresh water. It would appear that a much less profound physiological change would be required to transmute a sea water jelly fish into a jelly fish adapted to inhabit brine than would be required to enable it to inhabit fresh water. Yet the latter is the direction in which the modification has taken place, and taken place so completely that the sea water is now more poisonous to the modified species than is fresh water to the unmodified. There can be no doubt that the modification was gradual — probably brought about by the ancestors of the fresh-water medusa penetrating higher and higher through the brackish waters of estuaries into the fresh waters of rivers, and it would, I think, be hard to point to a more remarkable case of

profound physiological modification in adaptation to changed conditions of life."

In the summer of 1905, as already pointed out, this medusa appeared in considerable numbers in the aquaria of the Botanical Garden of Munich. In a recent paper (*Zeits. f. allgemeine Physiol.*, Bd. VII.), Maas has recorded a series of experiments made to test the reactions of the medusæ to various stimuli, chiefly chemical and mechanical, with brief references also to the influence of temperature. Experiments made with physiological salt solution confirmed in a general way those of Romanes already considered. For example, specimens when first placed in the solution showed quickened, spasmodic and heterochronous contractions, followed later by collapse and finally by death. If left in the solution for only ten minutes and then returned to the normal water the irregularity gradually disappears and the pulsations become synchronous and normal. That these reactions were not due to any difference in temperature he proves by control experiments made on various specimens at the same temperatures in fresh water, and even in water at very much lower temperatures.

He also tested the reactions of emarginated specimens in the salt solutions and found the same spasmodic contraction as observed in the uninjured specimens. A brief summary of the comparative effects of KCl and NaCl is given, from which it would appear that the former tends to inhibit reactions, while the latter serves as an active stimulus, though of a very irregular or heterochonous character.

Maas also points out the somewhat conflicting results of Loeb's experiments on *Gonionemus* and *Polyorchis*, those of Bethe on *Olindias*, and Herbst on *Obelia*; and concludes that among marine medusæ there must be essential differences in their chemico-physical relations.

In the transfer of a delicate creature, such as medusæ, from a medium like sea water to that of fresh water it might naturally be expected that reactions more or less remarkable should occur. But what shall be said of similar reactions which follow the transfer from a fresh-water medium, where if any soluble salts be present it must be in homœopathic doses of high attenuation,

to distilled water devoid of any trace of these elements? Surely it can hardly be claimed here that continued rhythmic action, or its inhibition, is attributable in any specific way to the ions of *this*, *that*, or *any* sort! Possibly those who would vindicate the ion hypothesis at all hazards might assume, as a desperate measure, that certain infinitesimal quantities of these elements may have been carried over in the tissues of the medusæ in their transfer from the normal to distilled water! But the assumption is so wholly gratuitous as to preclude serious consideration.

The writer has no theoretical views to promote or defend. But face to face with the facts he assumes, what is the right of every investigator, namely, freedom to discard any hypothesis which fails to account for his facts, and in so doing the further duty of exposing its futility. A comparison of the experiments heretofore set forth, and those to which direct reference has been made, together with others of similar nature and involving similar methods, shows such degree of confusion, not to say absolute contradiction, in the results obtained, as to suggest pause and serious reflection before any hasty leap be made in formulating conclusions. There is an old, but reputable, saying though involving something akin to paradox: "If the light that is thee be darkness, how great is that darkness." In the light of existing darkness concerning the problem under consideration might not some modium of scientific modesty and hesitation well replace something of the arrogance and dogmatism which have bulked so large in recent literature of these problems?

SYRACUSE UNIVERSITY,
February 25, 1908.

AN UNUSUAL GRAAFIAN FOLLICLE.

ELLISON A. SMYTH, JR.

In the fall of 1906 I became possessed of a young setter pup, with an interesting family history: her mother a rather small Gordon setter, had given birth to her first litter, to which my pup belonged, in June, 1906, which litter consisted of fourteen pups, four dogs and ten sluts. Two died when three or four months old, and the rest were successfully raised. The mother had seven tits in lactation.

For reasons of convenience I had this pup spaed in March, 1907, she being then ten months old. The ovaries I killed at

FIG. 1.

once in Flemming's solution and sectioned for class use. They showed a number of ripe follicles, not a few containing double and triple ova, though not all the extra ova were normal. Several serial sections on one slide, however, showed a large follicle

about ripe with two perfect ova at opposite poles; in each of these ova the nucleus was normal and the chromatin elements and network and also nucleolus perfect and beautifully distinct. Unfortunately I partially destroyed this slide through a vexatious accident. I obtained, however, another pair of ovaries from another pup, and on sectioning found many follicles with two, a number with three, a few with four, and one follicle with seven ova in clear view. As far as I can ascertain this is the greatest number of ova recorded from any mammalian follicle. The case is especially interesting on account of the family history. The mother setter has never had another litter. One of her daughters littered in July, 1907, having nine pups, three dogs and six sluts, with eight tits in lactation.

These facts are interesting in their suggestiveness along heredity lines, and also as to the relation between multiple ova in follicles, and large number of offspring.

The micro-photograph is of this follicle of seven ova,

VIRGINIA POLYTECHNIC INSTITUTE.

UNIVERSITY OF ILLINOIS
MAY
1908

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BIOLOGICAL BULLETIN

AN INTRA-NUCLEAR MITOTIC FIGURE IN THE PRIMARY OÖCYTE OF A COPEPOD, CANTHOCAMPTUS STAPHILINUS JUR.

ROBERT W. HEGNER.

The oögenesis and early development of the eggs of free living copepods has been studied by several investigators principally Häcker and Rückert. The former ('92, '95) has described the maturation processes in *Cyclops*, *Canthocamptus* and several other species. Rückert ('94) studied the polar-body formation in *Cyclops*, *Heterocope* and *Diaptomus*. The development of the eggs of parasitic copepods has also received the attention of embryologists (McClendon, '06).

As far as I have been able to learn none of these investigators has reported the presence of polar rays in the mitotic figure of the first maturation division. Rückert ('94) maintains that the centrosomes of the first maturation spindle (*Cyclops*) have an intra-nuclear origin; they pass to either end of the nucleus, but no polar rays were reported. Häcker ('95) in *Canthocamptus staphylinus* figures a spindle with chromosomes arranged in an equatorial plate lying entirely within the nuclear membrane of an oögonium which is undergoing its last division. Here also no astral fibers are evident. Parasitic copepods apparently do not depart from this rule for McClendon ('06) tells us that in *Pandarus sinuatus* the first maturation figure is "similar to that found in free living copepods having no polar rays." Centrosomes originate within the nucleus in the spermatocytes of *Ascaris megalocephala*, var. *univalens* (Brauer, '93) but wander outside of the membrane before the astral fibers appear. This is apparently the order of events in the half-dozen other cases where an intra-nuclear origin of the centrosomes has been asserted.

While collecting *Hydra* with Mr. C. T. Vorhies during the month

of January, 1908, a number of copepods was found scattered about in the vegetation which was gathered from the rocks on the bottom of Lake Monona, Madison, Wisconsin. These were identified as *Canthocamptus staphylinus* Jur.[1] I sectioned some of them and found a number of oöcytes in process of division. A few of these contained mitotic figures which possessed distinct polar rays at either end of the spindle, and were entirely enclosed within the nuclear membrane. This, I believe, is the first instance on record of such a phenomenon in the cells of either plants or animals.

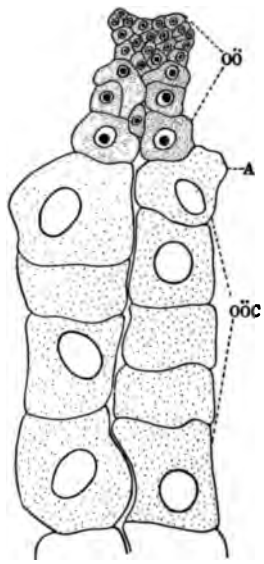

FIG. 1. Partially diagrammatic drawing of a longitudinal section through the two oviducts of *Canthocamptus staphylinus* Jur. *a*, oöcyte shown enlarged in Fig. 2; *oö*, growing oöcytes; *oöc*, primary oöcytes.

The ovaries of *Canthocamptus* lie in the dorsal cephalic region. The two oviducts extend posteriorly from them lying on either side of the median line of the body; each when filled contains a single row of eggs. The primary oöcytes can readily be distinguished from the growing oöcytes by the different staining capacity of their yolk laden cytoplasm. In the case of the latter the cytoplasm stains very deeply in hematoxylin, while that of the eggs in the processes of maturation is much less susceptible to this dye. An abrupt change in the chemical character of the cell contents may thus be recognized where the growing oöcytes end and the primary oöcytes begin (Fig. 1).

In the present paper we shall consider only the primary oöcytes in the equatorial plate stage of nuclear division. Several hundred females of *Canthocamptus* were sectioned, but only two of these contained eggs showing mitotic figures. Twenty-two of the eggs examined were in process of division; in each one, the entire amphiaster was found within the nuclear membrane. One of these eggs (Fig. 1, *a*) is shown enlarged in Fig. 2. The chromosomes have been drawn up about the spindle and form the equatorial

[1] I wish to thank Mr. C. Dwight Marsh for determining the species for me.

plate of the first polar mitotic figure. The number of chromosomes was not determined. The nucleolus, which stains very deeply in the growing oöcytes, is lighter in the primary oöcytes, and often has several vacuoles within it. In a few cases two nucleoli, one larger than the other, were found in a single nucleus. Fig. 2 shows the various cellular structures as they appear when magnified 850 diameters.

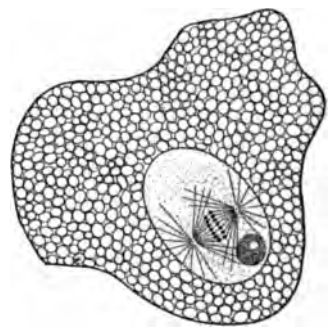

FIG. 2. A single oöcyte of *Canthocamptus staphylinus* Jur. enlarged from Fig. 1, *a*. The entire amphiaster lies within the nuclear membrane. × 850.

A discussion of the above described condition in its relation to amitosis and other nuclear phenomena is deferred in the hope that material collected later in the season may furnish stages which will enable me to follow the history of the formation and the fate of this unique mitotic figure.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF WISCONSIN,
March 13, 1908.

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A MICROSCOPE-STAGE INCUBATOR

J. THOS. PATTERSON.

The desirability of studying the living egg in connection with any embryological investigation has long been recognized, and the excellent results obtained from such studies have demonstrated repeatedly the advisability of putting forth every effort to overcome any obstacles that might stand in the way of such observations. Hitherto direct observations on the developing bird's egg have not been possible, for such a study is beset with many difficulties, chief among which is that of incubation. In an endeavor to overcome this difficulty I have been led to devise the following *microscope-stage incubator*, which not only fulfills my purpose admirably, but also gives promise of being useful in the study of other biological problems, especially those in which it is necessary to maintain a constant temperature while making direct observations on the living organism.

The photograph (Fig. 1) shows the apparatus connected up and ready for use with a compound microscope although a binocular can be used as readily. The incubator consists of a galvanized-iron tank, a portion of which fits over and in front of the microscope stage. Just above the center of the stage a hole is cut in the lid of the tank and in this is placed a covered dish, or egg-cell *e*. This arrangement allows one to study the egg readily and at the same time to make camera drawings of the object under observation.

The water in the incubator is heated by a sixteen-candle power incandescent lamp *l* connected with a thermoregulator *r*, which is patterned after the glycerin type described by Mast (*Science* for October 25, 1907). The bulb or immersed part of the regulator is bent at right angles to the upper portions, in such a way that it extends towards the microscope stage, reaching almost as far as the egg-cell. This arrangement not only places the bulb directly in the path of the current which flows from the

incandescent to the egg-cell, but also increases the size of the bulb, and thus increases the sensitivity of the regulator.

For my purpose, the regulator is adjusted so as to maintain a temperature of about 39.4° C. in the neighborhood of the thermometer *t*, which is placed as close to the egg-cell as will permit an easy manipulation of the objectives. In order to keep the temperature constant it is necessary to have a circulation of the

FIG. 1.

water within the incubator, and the way in which this is accomplished can be understood better from the drawing (Fig. 2), in which the greater part of the top and back has been cut away in order to show the internal structure. The shaded part of the drawing represents that portion of the incubator that fits about the microscope stage. For convenience we may speak of three apartments, *A*, *B*, and *C*. *A* is separated from *B* and *C* by a

partition in which are two openings, *m* and *n*. The regulator bulb passes through the former of these holes. In the first apartment the water is heated by the incandescent at *X*; in the second is the egg-cell; and the third is the passage for the water from *B* to *A*. The arrows indicate the direction of the main current of water, and the principle by which this current is maintained is simple. Thus the water on being heated at *X* spreads out over the top of apartment *A* and enters *B* through opening *m*, and consequently flows past the thermoregulator bulb and around the egg-cell. Apartment *C*, in addition to being that portion of

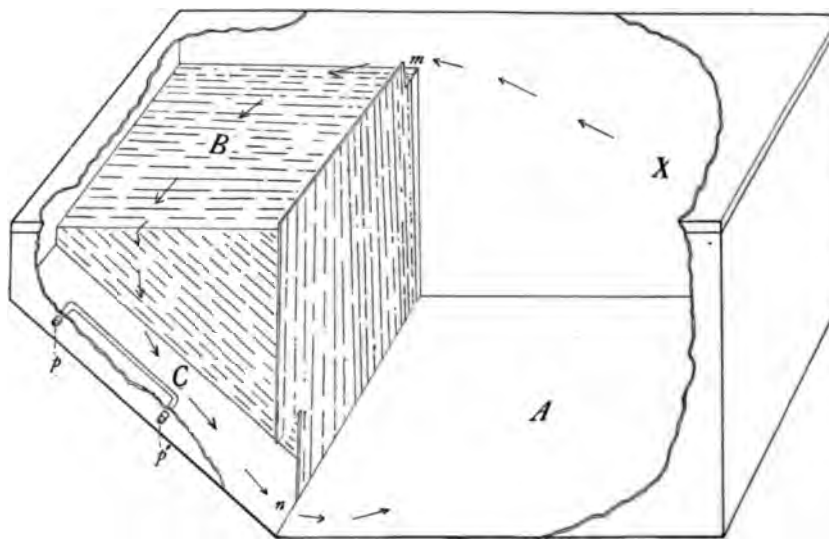

FIG. 2.

the incubator situated farthest from the source of heat, is so constructed that a considerable portion of its surface is exposed for radiation, and hence, the water in this chamber is gradually cooling, and in so doing will flow back through *n* into *A*. It should be added that the lower layer of water in *A* is also heated by the incandescent, at least sufficiently to raise it to a higher temperature than that in the lower part of *C*, and consequently the water in this latter region passes into *A*. At the same time the upper layers of *C* in turn cool and sink. There is maintained throughout the three apartments, therefore, a constant current,

which, although imperceptible, is yet capable of demonstration; for by holding the bulb of a delicate thermometer in the various parts of the incubator the different degrees of temperature are clearly indicated. I have found it advisable to immerse only about a half or two thirds of the incandescent lamp, for in this way the circulation of the water is greatly facilitated.

In constructing the tank, a small pipe (p–p') was soldered into the side of C, so that in case the water did not cool here with sufficient rapidity, a cold stream from a tap or reservoir could be run through the pipe, thus insuring a constant current throughout the three apartments. This precaution was later found unnecessary, at least for temperatures at which the bird's egg incubates.

When the incubator is arranged as described above the temperature of the water in the region of the egg-cell does not vary over 0.2° C., a variation practically negligible for all ordinary purposes. However, if it be desired to maintain a temperature even more constant than this, it could easily be done by making the regulator more sensitive in the ways suggested by Mast, and by constructing the tank so as to prevent the loss of heat by radiation.

This tank, which measures thirteen inches long, seven wide, and six and a quarter high, can be made by any good tinsmith, and the apparatus with all its fixtures can be had at a cost not to exceed five or six dollars.

UNIVERSITY OF CHICAGO.

THE GENESIS OF FAULT-BARS IN FEATHERS AND THE CAUSE OF ALTERNATION OF LIGHT AND DARK FUNDAMENTAL BARS.

OSCAR RIDDLE.

With 4 Plates and 5 Figures in the Text.

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INTRODUCTION.

During recent years several attempts have been made to find a basis, structural or physiological, for the color patterns of various animals. Among the most notable and successful of these

may be cited the studies of Graf[1] on leeches and of Loeb[2] on fish embryos. Graf was able to show that the characteristic markings of the leeches examined depend primarily upon the number and arrangement of the muscles of the body-wall. Loeb showed that in *Fundulus* embryos, markings are produced by a grouping of pigment-cells around the blood-vessels. The present paper is chiefly a report of observations and experiments which furnish a physiological basis for what seems to be the primitive color-markings of the feathers of birds.

This research was undertaken at the suggestion of Professor C. O. Whitman; it is here a pleasure to thank him for much kindness and encouragement and to acknowledge the great value of his help and criticism.

Two observations by Professor Whitman furnished the starting point for these studies; first, there is in all feathers a "fundamental barring" of the whole length of the feather; second, certain defects (fault-bars) occasionally appear in the plumages of birds reared under adverse conditions. In presenting the facts which furnish a physiological basis for these two characters, it seems best to consider the fault-bars first, since they furnish the key to an understanding of the fundamental bar.

The Morphology of Fault-Bars.

I have found five types of defects and have made it certain that they all really represent the same thing. Two of these types were seen and described by Strong[3] from a hybrid pigeon. Duerden[4] has also reported one of the types described by Strong as occurring in great numbers in ostriches. These defects have doubtless been seen by several naturalists. Darwin came across them in at least two instances. He cites[5] some variations in the

[1] Graf, Arnold, "Ueber den Ursprung des Pigments und der Zeichung bei den Hirudineen," *Zoöl. Anz.*, XVIII., 1895.

[2] Loeb, Jacques, "A Contribution to the Physiology of Animal Coloration," *Journal of Morph.*, Vol. VIII., 1893.

[3] Strong, R. M., "A Case of Abnormal Plumage," Biol. Bull., Vol. III, November, 1902.

[4] Duerden, J. E., "Bars in Ostrich Feathers," *Agr. Jour.*, Cape of Good Hope, May, 1906.

[5] Darwin, Charles, "The Variation of Animals and Plants under Domestication," Vol. I., p. 267.

hackles of a sub-variety of the game-cock in which "the tips having a metallic lustre are separated from the lower part of the feather by a symmetrically shaped, 'transparent zone' composed of the naked portions of the barbs." In another work[1] he describes the "transparent zone" of the ocellus of the peacock's

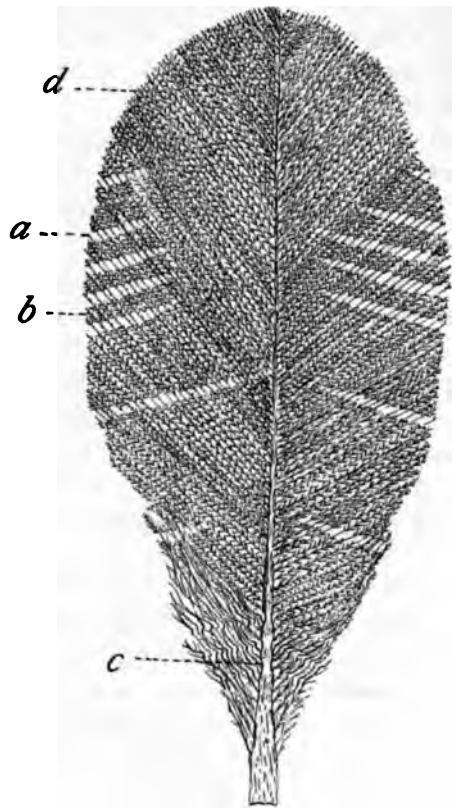

FIG. 1. Feather from a poorly nourished chick showing abnormalities. *a*, abnormal area; *b*, "fundamental bar" (a day's growth); *c*, constrictions; *d*, region in which defective lines showed plainly in this feather. × 2.

feather. Both of these belong to the sort of structure now under consideration.

Since so little has been said of these defects, and since their significance has nowhere been recognized, it seems advisable to give a very complete and detailed description of them. Such a

[1] Darwin, Charles, "The Descent of Man," 1871.

erest conditions which a bird can encounter and endure. In types 1 and 2, the barbs and shaft are often bent or kinked in the abnormal region.

The third type of defect is something very much less conspicuous than either of the two types just considered. It cannot be represented in a drawing. It is a very minute depression or differentiation of some sort extending across the surface of the feather in exactly the same direction as do the defective areas of type 1. It is not always easy, however, to determine that it is a depression at all. It often seems to be a line, or simply a point of union of the distal with the proximal part of the feather-vane. This line is sometimes so inconspicuous that even close observation may not reveal it. It is probable that these lines are not always depressions; but, that differences in light-reflecting power exist between a point in such a line and points anterior and posterior to it, is unquestionable.

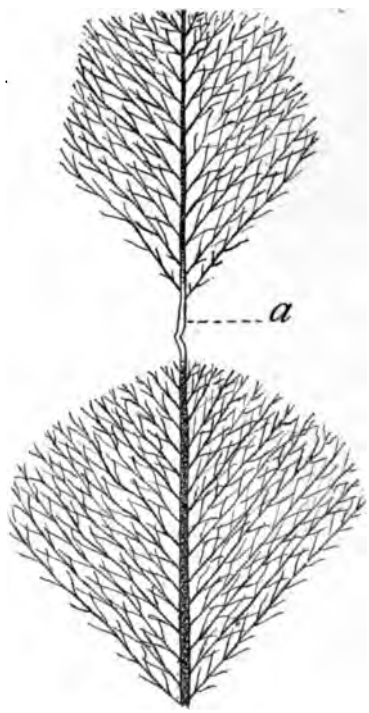

FIG. 2. Abnormal region of a plume from an ostrich chick (kindly sent to me by Prof. Duerden). *a*, fault-bar, type 2, in which shaft only is present. This region shows also a great reduction of the pigmentation of the shaft. × 4.

The term "line," is moreover, not a thoroughly satisfactory one, for, that these defects intergrade with others of appreciable *width* is certain. These lines are thoroughly characteristic of the feather and are properly classified among feather defects, for it is at such joints that defects like those of types 1 and 4 appear.

Many feather-vanes show at certain points in their length very deep depressions or constrictions which give to them a wavy appearance. At first sight such modifications have nothing in common with types 1 and 3, but closer study proves that they

that one sees all possible intergradations, that each marks off a day's growth, that when the area of type 1 occurs it always falls in the place for the line, that a certain part of the line only may be transformed into the obviously defective area, etc. Type 5 is probably caused by a protracted defective nutrition of a segment of the circle of growing feather-elements in the germ.

In connection with these fault-bars we may mention another condition met with in some extreme cases in which abnormalities extend almost continuously over several millimeters of the feather's length. There occurs not only a weakening of the parts, but also a lack of differentiation of the feather-elements. This is well shown by feathers from the nape of a chick which had been fed with Sudan III. In this case the inner and outer sheaths failed to separate from the barbs, and a banded condition results. Another example of such a structure grown in nature by an English sparrow is shown in Pl. XIII., Fig. 11. The lack of differentiation, curiously enough, occurs in only two rectrices and these were corresponding ones on opposite sides of the tail. Pl. XIII., Fig. 12, shows also an adjoining rectrix. The latter betrays by its similarly placed fault-bars the fact that they were both produced by the same cause. I have found similar bands on feathers from the head of the cardinal. They were being produced simultaneously with deep constrictions in the other feathers of the bird.

The Feather-germ. — The more prominent defects have been observed in the feather-germ, both in their initial stages and immediately before the breaking away of the containing sheaths and the unfolding of the feather-elements. The less conspicuous defective lines (type 3) have, however, escaped all observation in the germ, as indeed they have done in the adult feathers when the microscope was the means of observation. This apparently means that the modifications of such regions are extremely slight, not localized with extreme sharpness, and consist chiefly in slightly different powers to absorb or reflect light; this difference in reflecting power being better seen when the field of vision is large and the contrast between the parts of a large area plays a part.

The appearance of extreme constrictions on an expanded feather-germ has already been cited. The further fact that at

the constrictions in unexpanded germs the epidermal layers invade the parts normally occupied by the dermal pulp has also been referred to (Pl. XII., Fig. 3). Only another word concerning the histology of the defects need be said at this point, and the subject will be more fully discussed along with the cause of the defects. The normal development and histology is too considerable a subject to be considered here and the reader is referred to the papers of Studer,[1] Waldeyer,[2] Davies,[3] Haecker,[4] Maurer,[5] Strong[6] and others[7] for this information.

Much of feather structure is indicated in the plates of the present paper, but the description will deal only with such structures as are directly concerned with our own problem. The general relations of the parts of a feather in cross-section are shown in Pl. XII., Figs. 2 and 5.

The fault-bars in their earliest stages are indicated by a loose union of scattered cells in that part of the *intermediate* cell-layer which is forming the barbule cells. The cylinder-cell layer and apparently the more central cells of the intermediate cell layer (*i. e.*, those last formed from the cylinder layer) are crowded together as usual. Pl. XV., Fig. 26, represents some cellular relations at the close of a fault-bar producing period.

The Extent and Distribution of the Fault-Bars.

It has been stated that Professor Whitman was the first to direct attention to these abnormalities. He had observed them in some of his pigeons; one of these birds, a hybrid, furnished the material for Strong's[8] description of two types of defects

[1] Studer, T., "Die Entwicklung der Feder," Inaug.-Dissert., Berne, 1873. "Beiträge zur Entwicklungsgeschichte der Feder," *Zeit. f. wiss. Zoöl.*, Bd. 30, 1878.

[2] Waldeyer, W., "Untersuchungen ueber die Histogenese der Horngebilde, insbesondere der Haare und Federn," *Beiträge z. Anat. u. Embry. als Festgabe J. Henle*, Bonn, 1882.

[3] Davies, H. R., "Die Entwicklung der Feder und ihre Beziehungen zu andern Integumentgebilden," *Morph. Jahrb.*, Bd. 15, 1889.

[4] Haecker, V., "Ueber die Farben der Vogelfedern," *Arch. f. Micr. Anat.*, Bd. 35, Heft 1, 1890.

[5] Maurer, F., "Die Epidermis und ihre Abkömmlige," Leipzig, 1895.

[6] Strong, R. M., "The Development of Color in the Definitive Feather," *Bull. Mus. Comp. Zoöl. at Harvard*, Vol. XI., 1902.

[7] A very satisfactory bibliography to 1902 is given by Strong.

[8] *Loc. cit.*

already referred to. While this part of the present work was well on the way toward completion Professor Duerden[1] reported the "bars" (these are the exact equivalent of fault-bars of type 1) in the ostriches, and is at present attempting to rid those birds of them. He estimates that the value of the ostrich plumes from South Africa alone, are, by the presence of these defects, depreciated in value to the extent of £250,000 annually.

In the Bird Group. — The defects, however, are not confined to hybrid pigeons and domesticated ostriches. By simply looking for them it has been easy to find them everywhere. The *pronounced defects* of type 1 have been seen in parrots, trojans, owls, motmots, kingfishers, cuckoos, humming-birds, penguins, hornbills, turkeys, doves, chicks, English sparrows, herons, gulls, bluebirds, cardinals, robins, flamingoes, pheasants, loons, pea-cocks, etc.; everywhere, indeed, that I have looked for them except in fossil feathers, artists' drawings, and journals of ornithology! It will be seen that the defects occur in widely separated bird groups; in primitive and in recent birds; in land and water birds; in domesticated and in wild birds; in birds from the arctic and from the torrid zone, etc. I have been able, owing to the courtesies extended by Professor C. B. Cory and Dr. Ned Dearborn, of the Field Museum of Natural History in Chicago, to examine a very great variety of birds belonging to the Museum. I find that although it is not easy to see evident defects (*i. e.*, broad defective areas) in every specimen, it is easy to find them in every species. We may conclude, therefore, that they are to be found in *all* birds.

It is a fact, and a significant one I think, that the defects are, in general, more common in domesticated and caged birds than in wild birds. In this connection, however, it should be stated that the defects appear indifferently in pure breeds, hybrids and mongrels. At any rate I have verified this in a number of our domesticated birds. The effects of "inbreeding" have not been observed.

In the Various Plumages and Pterylæ. — I have found the emphasized defects in all of the plumages of birds, with the exception of the first or downy plumage. In some birds the

[1] *Loc. cit.*

defects seem to occur more frequently in the juvenal plumage (of Dwight) than in the others.

Evident defects appear in all the feather-tracts or pterylæ; but in a particular bird, and usually in a particular species, certain tracts show them in greater numbers than do others. In the ring dove, *Turtur risorius*, the order of frequency of occurrence is: rectrices, remiges, wing coverts, etc. In *Gallus* the order is: remiges, rectrices, wing or body coverts, etc.

In an Individual Feather. — In the feather there may be produced at any point in its length, either of the five types of abnormality. In some birds (*Gallus*) the distal part of the feather oftener shows the defective areas; the proximal end, the deep *constrictions* (type 4), while we get defective *lines* (type 3) in one form or another at all points in the feather's length.

The recognition of weakened areas as universal in feathers throws a new light on the rather over-discussed subject of feather *abrasion*. That there are birds whose feathers "normally" have the barbules broken off at certain fairly definite points in the more distal barbs has been observed by Meves,[1] Chapman,[2] Dwight[3] and others. Meves and Chapman have noted, too, that the barb itself may be broken near the distal end. I have seen several cases among wild birds of the breaking of a series of barbs at the point where they were crossed by the same defective line, and am convinced that further study will prove that most feather abrasions occur by the breaking away, as a single piece, of that portion of a barb which occupies the space between two fault-bars. That such breaks do occur at the fault-bars I have often proved by pulling the distal end of a series of barbs and noting the point at which they break. A feather treated in this way is shown in Pl. XIII., Fig. 7.

[1] Meves, W., "Über die Farbenveränderung der Vogel," *Jour. für Ornith.*, Bd. 3, 1855.

[2] Chapman, F. M., "On the Changes of Plumage in the Snowflake," *Amer. Mus. Nat. Hist.*, Vol. 8.

[3] Dwight, J., Jr., "The Sequence of Plumages and Moults in the Passerine Birds of New York," *Ann. N. Y. Acad. Sci.*, Vol. 13, No. 1.

The Experimental Production of Fault-Bars.[1]

Professor Whitman's suggestion that fault-bars are due to the malnutrition of the feathers during their growth, was put to a direct and vigorous experimental test. The method employed varied with the nature of the experiment and with the material, which was of four kinds. Of course, one had to work with *growing* feathers. Those experimented upon were (1) the juvenal feathers of ring doves (*T. risorius*); (2) the later plumages of ring doves produced at the regular season of moult; (3) feathers, chiefly remiges and rectrices, of ring doves obtained at any season by previously removing some of these; (4) juvenal and adult plumages of chicks (*Gallus*). All of these yielded entirely comparable results; the young birds merely showing a greater sensitiveness to lack of food. Five types of experiment were tried: (1) Reduced feeding or starving; (2) feeding with Sudan III.; (3) mechanical crumpling of the germs; (4) effect of light, temperature, bad sanitary condition of the nest, parasites, etc.; (5) amyl nitrite.

Reduced Feeding. — To reduce the feeding of the young doves one had only to limit the feeding of the parents, the latter refusing to regurgitate the food for the young when insufficiently fed. By this means one could not be certain of the amount eaten by the young unless the parents were not fed during a couple of days. In many of these cases the young died. In those cases where a partial starvation of the young was evident, one invariably found later, one or more fault-bars to correspond to it. In some cases the old birds were fed normally and one of their young was left with them as a control while the other bird was placed either in an incubator or under other nesting birds. The experimented bird could then be replaced with its parents and fed by them as little or as much as the experiment demanded. Twenty-four hours without food was invariably accompanied by the production of pronounced fault-bars (of type 1) in these birds. The control birds usually did not show these obvious defects (Pl. XIII., Figs. 6 and 7).

[1] Incomplete results of these experiments were communicated (1905) to Professor Duerden, who in his paper already cited (1906), wrongly credits the work to Drs. Strong and Whitman.

When adult ring doves were starved for periods of one to three days, *those portions of the feathers grown during those days showed well-marked fault-bars, one* (exceptionally two) *for each day of growth.* In such experiments the length of the feathers was measured at the beginning and again at the end of the starving period. For this experiment a control bird of similar age and condition was kept and fed normally in a cage alongside the starved bird. Measurements of the feathers of the control being taken also. The effect of three grades of feeding on the rectrix of a dove is shown in Pl. XIII., Fig. 6.

Many experiments have been made upon feathers which were replacing others that had been purposely removed. In these cases, the rectrices were pulled on the same day from two or more similar birds. After their new feathers had started to grow they were divided into experimental and control birds, and the low-feeding commenced. In Pl. XIII., Fig. 6, the effects of several days of starvation on such a feather have just been noted. This feather shows an absence not only of most of the *barbules* of the affected region but of the distal ends of the *barbs* as well. Careful inspection showed an occasional defective area in the control also.

The effects obtained by starving chicks — old or young — are in every way comparable to those just stated for doves, and a separate description therefore need not be given. Any considerable reduction of the food of doves and chicks will invariably produce well-marked fault-bars in many of their growing feathers.

The Feeding of Sudan III. — While the fat stain, Sudan III., was being fed to some young chicks for a quite different purpose, these were found to be producing defects similar to those produced by starving. This led to a careful study of what the stain could accomplish in the way of producing these abnormalities. *As a result of this study it can be said that when Sudan is fed in large amounts fault-bars are laid down in much the same manner as in the starving experiments.* The chief difference being that with Sudan the defects much more frequently take the form of constrictions (type 4) than of defective areas (type 1). Examples of the latter type are not uncommon, however, and were even large enough to appear in photographs (Pl. XIII., Fig. 13). That the

Sudan probably acts by reducing the actual nutrition of the bird is a conclusion that will be referred to again.

Birds for these experiments were kept under similar conditions in the three compartments of a specially constructed brooder. One lot of chicks served as control; another was fed a small amount of the stain; another a maximum amount. The number of fault-bars produced in any feather stand in this order (the number of feathers grown by the birds in the reverse order). The stain was fed in creamy milk (all the birds were given the milk). It was found that one had to feed the stain with the milk at the first time the milk was offered; otherwise the birds avoided it.

Effect of Mechanically Crumpling the Feather-germs. — In carrying through the experiments already described, a considerable amount of handling of the birds was unavoidable. It, therefore, seemed necessary to learn whether any of the defects, and particularly the few showed by the control, could have been produced by this procedure. This was tested by slightly marking in various ways one of the two birds of a brood; the marked bird (or in other cases the unmarked one) was then occasionally taken from the nest, its feathers measured, etc., as had been done in the earlier experiments. It was found that *this ordinary treatment was not followed by the production of evident defects.* When, however, the feathers were *strongly crumpled or broken in the region of growth, fault-bars resulted,* and by this means it was easy to *produce diffuse fault-bars at all levels of the feather* and this *quite independently of the usual time element involved.* That is to say, these large defects were laid down at irregular intervals, the space between two groups of defects depending upon the frequency with which the germs were crumpled. Crumpled primaries from the right wing are shown along with the corresponding ones from the left wing of a ring dove in Pl. XIII., Figs. 20–21.

General. — Several experiments of various kinds were next tried in order to learn whether the defects were in any way related to other conditions attending the birds in their nests. To this end, one of a pair of young birds were repeatedly taken from the nest and left exposed to cool air more than was the other; some birds were reared in foul nests, others in clean ones; some birds were infected with bird-lice, others not, and so on through the

range of factors which might conceivably be acting on the birds. *The results seem to fully justify the statement that none of these conditions can account for the defects*, neither in those of the experimented birds nor the occasional ones of the control. The net result of all the experiments thus far served apparently to demonstrate that the important factor in the production or non-production of the emphasized defects is *nutrition*. It did not, however, seem to be the only factor, for defects might sometimes be seen in feathers of well-fed birds. The difficulty thus presented was largely cleared up by the progress of studies in another direction (discussed under the next division of this paper). These studies had made it certain that normally *one defect is laid down in the germ for each day of growth;* while the examination of the various types of fault-bars had made it certain that the defective area of type 1 is a true representative of the defective line of type 3. The latter is, therefore, laid down daily as is the defective area. It follows that the proof of a causal relation between nutrition and *defective areas* is at the same time a proof that nutrition is causally related to the *defective lines* (that types 1 and 3 are merely different forms of the same defect I have already shown). And since the latter are present in all feathers from tip to tip, one for each day of growth, it is evident that the efficient cause (*i. e.*, nutrition) acts rhythmically. The few defective areas in control feathers could then be due, conceivably, to a slight emphasis of this normal internal rhythm. Since nutrition is a proved factor for the most extreme defects, it seemed extremely probable that it was also playing the chief part in the formation of all types of defects, including the extremely faint, elusive, and universal, defective lines. From this it would appear that the internal rhythm is a daily rhythm, and that it is able — like my experiments — to interfere with the nutrition of the feather-germ. This suggested *blood pressure* to me. To test this idea the experiments described in the following section were undertaken.

The Production of Fault-bars with Amyl Nitrite. — It is well known that amyl nitrite powerfully reduces the blood pressure in mammals. Some preliminary experiments on the effects of amyl nitrite on the circulation of the chick showed that when traces of it are inhaled, an immediate and extensive vaso-dilatation occurs.

Both the arterioles and veins of the comb, wattles, patagium, etc., are dilated. The strongest action, however, is exercised here as in mammals [1] upon the vessels of the viscera. This was ascertained by making considerable incisions through the body-wall so as to allow free observation; this was quickly followed by giving the drug. In other cases the bird was anæsthetized with ether before exposure of the viscera. The action of several drugs on the blood-pressure of birds has been studied by Dr. S. A. Matthews and the writer.[2] One of our tracings, showing the effects of amyl nitrite on the vascular pressure of a duck, is shown in text Fig. 5. A glance at this figure is sufficient to convince one that the

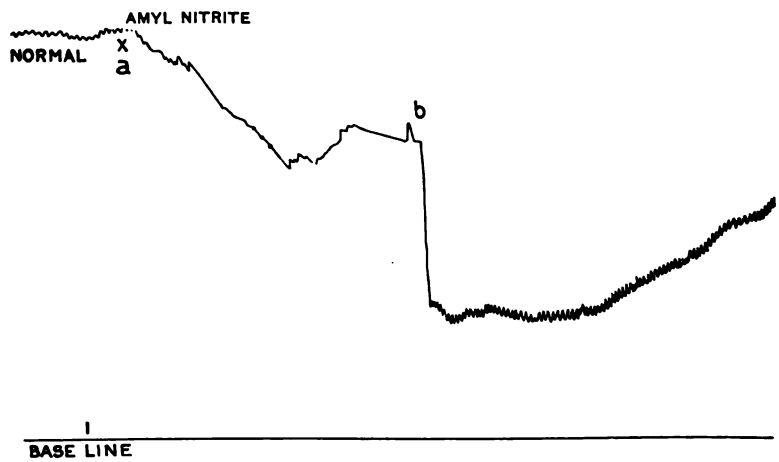

FIG. 4. Blood pressure tracing from the carotid artery of a duck. A normal pressure of 164 mm. Hg is here recorded. Amyl nitrite was given at *a* and again at *b*. The tracing shows a rapid fall in the arterial pressure to 50 mm. Hg.

alleged powers of amyl nitrite to diminish blood pressure is no myth.

To test directly the efficacy of amyl nitrite in the production of fault-bars the experiment was carried out in the following way: Two Plymouth Rock chicks of equal age (7 months) were taken at the time of their first moult; on the evening of the first experiment the distal ends of a number of their freshly expanding feathers were measured and *very carefully* cut away at a distance

[1] Cushny, C. A., "Pharmacology and Therapeutics," p. 470, Philadelphia, 1899.

[2] Riddle, O., and Matthews, S. A., "The Blood Pressures of Birds and Their Modification by Drugs," *Amer. Jour. of Physiol.*, Vol. XIX., June, 1907.

of 18 mm. from the skin. At 8:00 P. M. the birds were brought into the laboratory and were placed in similar large glass jars; a tube of amyl nitrite was broken in a bottle, the bottle was partially stoppered and placed in the jar with chick No. 1; the dose was regulated by means of the stopper; the comb and patagium serving as indicators. On the first night, however, in order to make sure that it was effective, the dose was increased until the bird toppled over unconscious; the bird was taken out and revived, replaced, and the supply of amyl nitrite slightly reduced. The bird now showed no discomfort, but chuckled and sang as if to express satisfaction; the birds were observed until 12 P. M. At 8:00 A. M. they were both released, returned to the green-house and fed as usual; at 8:00 P. M. of the same day they were again brought into the laboratory and the procedure of the previous evening repeated; at this time, however, No. 1 was not given sufficient of the drug to produce unconsciousness; on the following day at 8:00 A. M. they were again returned to the green-house; measurements of the rate of growth of some of their marked feathers were taken at intervals of two or three days during the next two weeks; feathers for sectioning were removed from birds I. and II. before the experiment began, and on the mornings following each experiment.

The result of this experiment leaves, I think, no room for doubt on one or two important points. When the parts of the feathers grown during the two days of the experiment had expanded, it was found that in No. 1, *two pronounced defects* (Pl. XIV., Figs. 22–23) *had been produced.* The feathers of No. II. were normal (Pl. XIV., Fig. 24). The sections tell the same story. The longitudinal section of a feather-germ (Pl. XV., Fig. 25) taken from the bird on the morning after the first experiment shows an abnormal region at about 2.5 mm. from the end of the feather. This undoubtedly represents the fault-bar produced on the preceding night.

There can be no question that amyl nitrite, when used as outlined above, is able to produce fault-bars in chicks. The question may of course be raised as to whether it does so by lowering the blood pressure or by some other means. In my opinion the probabilities that it does so are overwhelming, though its

capacity to form methæmaglobin in the blood and thus reduce the oxygenation of the tissues is a fact which should not be overlooked. But, if it be granted that a reduction by this means of the supply of oxygen to the tissues has a tendency to produce defects, then it becomes plain that *a reduction of the blood pressure alone must tend to produce them also*, since in any lowering of the blood pressure the amount of oxygen available to the tissues is decreased. It would seem moreover that we are justified in throwing out of consideration all other actions of amyl nitrite than those connected with the blood, since the inferior umbilicus of a feather-germ is a portal through which there enters from the body practically nothing except the blood-stream.

THE HOMOLOGIES OF THE FEATHER DEFECTS OR FAULT-BARS.

The conclusive proof which has just been given that the fault-bars of feathers are produced by a reduced nutrition puts us in a position to state positively that these defects are the homologues of other defective growths of epidermal structures long known to be thus caused. Among these may be named: imperfections of the enamel of the teeth (dental hypoplasia); the grooving of the nails after illness; certain changes in the hair, particularly the weakening of the fibers of the wool of sheep which had been underfed; and the rings on the horns of many ungulates. It has already been suggested by Duerden that these structures are similar in nature to the feather defects. The proof of the suggestion lies, however, as stated above, in the determination of the cause of the fault-bars.

There should be added to this list such similar markings of epidermal structures as the concentric rings of annual growth found on the shields of many tortoises, and probably also the stratified hoof-formations of many animals.

It is worthy of note that the range of time involved between two of these successive abnormal productions varies from one day to one year. In the feather, however, the time varies only from one day to several days — different types of fault-bars resulting from the different periods. There is, nevertheless, but one cause for all these various formations, namely, a reduced food-supply, and this warrants our grouping them together.

Of greater interest than these known cases of epidermic response to nutrition are, in my opinion, similar responses which have as yet not been recognized in other tissues and organs. The constantly observable daily effects on feather structure of only slight "normal" daily reductions of nutritive conditions in the bird has fully convinced me that similar, and longer starving periods, and seasons of hibernation "normally" passed through by many animals, *cannot but produce definite and lasting marks in many of the tissues and organs of these animals.* (The effects may often, indeed, be so slight as to escape observation even with the microscope, but this does not negative their existence.) The writer believes that from this standpoint it would be very desirable to study the formation and growth of many tissues and organs; particularly such lamellar structures as ivory tusks, bone, certain ova, pacinian corpuscles, and doubless many others.

Feather Growth.

It was recognized at an early stage of these studies that accurate data on the *region* and *rate* of growth in the feather must be secured. Very few of the numerous writers on feather structure and development seem to have concerned themselves with either of these problems. The region of growth has of course been indicated in a general way (most definitely in "down") but its very restricted limit has not, heretofore, I believe, been sufficiently emphasized. The figures of Davies,[1] Haecker[1] and the still better ones of Strong[1] are, however, suggestive of it. Some observations and experiments on the rate of growth in chicks and doves are reported here.

The Region of Feather Growth. — A study of the growing tips of remiges and rectrices of ring doves, and of the primaries of the chick, shows that the region which produces the cells which enter into the formation of the barbules is less in extent than that which enters into the formation of the barbs (Pl. XII., Fig. 1); the barbule producing region representing less than 1.5 mm. of the entire length of these large germs. This region apparently does not begin at the extreme end (inferior umbilicus) of the feather, but slightly above it, and surrounds the wide portion of the

[1] *Loc. cit.*

pulp cavity (see figures). Others have pointed out that the *differentiation* of the barbs occurs later, *i. e.*, at a higher level than that of the barbules. There must be added to that the further fact that some of the cells which enter into the former *arise later* (at a higher level) than those which form the latter. A few observations on feathers from various birds, together with Strong's[1] figures of *Sterna*, afford considerable reason for believing that these statements on the region of growth in the chick and the dove may have among birds a very wide application.

The Rate of Feather Growth. — The rate of feather growth has not, so far as I am aware, been extensively or accurately studied. Cunningham[2] found that certain tail feathers of Japanese fowls grow at the rate of 3.5 mm. per day. Ostrich plumes are said to grow about one inch per week. In Plymouth Rocks my measurements show a very similar rate of growth. The rate varies greatly in different feather tracts of the bird; for example, in a Plymouth Rock it was, in the primaries, secondary coverts and body coverts, 4, 2.25, 1.75 mm. daily respectively. In general, the rate bears a rather definite relation to the ultimate length of the feather; and is less at the proximal than at the distal end of the feather. This is shown in the figures (Pl. XIII., Figs. 17–19); these indicate also the presence of fault-bars which are laid down at distances corresponding to the figures given above.

The ring dove shows a still more rapid feather growth. *Seven mm. of growth in 24 hours* has occasionally been recorded in the rectrices of these birds. The average for these birds is: rectrices 5–6 mm., primaries 5–6 mm., upper tail coverts 4 mm., primary coverts 4 mm. It will be recalled that *this is also the order of frequency for the appearance of the defective areas in the various feather-tracts.* This and kindred observations establish beyond doubt that the frequency of appearance of obvious fault-bars in feathers is directly related — one might almost say proportional to the rate of growth. Table I. gives some figures on the rate of growth in doves.

The Effect of Starvation on the Rate of Growth. — Starving conditions when brought to bear on growing feather-germs pro-

[1] "Development of Color," *loc. cit.*

[2] Cunningham, J. T., "Observations and Experiments on Japanese Long-tailed Fowls," *Proc. Zoöl. Soc. of London*, 1903.

TABLE I.

Bird.	10	11	12	13	14	15	16	17	= Age of Feathers in Days.
									First 4 days = 6 mm. per day.
No. 83 starved	22	29	35	40	46	49	52	55	Last 3 days = 3 mm. per day.
									First 4 days = 6⅛ mm. per day.
No. 97[1] control	24	3	38	43	49	54	58	63	Last 3 days = 4⅜ mm. per day.
	Length of feathers in mm.								
									First 4 days = 4½ per day.
No. 20 starved	10	16	20	24½	28	32	34	36	Last 3 days = 2⅔ per day.
									First 4 days = 5 per day.
No. 100 control	12	17	22	27½	33½	39	44	50	Last 3 days = 5½ per day.

Showing rate of growth during seven days of starving and in control. The numbers in the first line at the top indicate the age of the feathers, *i. e.*, the number of days since the feathers of the previous plumage were removed. The first day of starvation is that between the tenth and twelfth days.

duce marked effects, some of which have been noted in connection with the production of fault-bars. It has been found that when the starving extends through periods of less than three or four days, that no diminution in the linear growth of the feather results (doves). The effects of such starvation are shown only, or at any rate principally, in that portion of the intermediate cell-layer which, as previously stated, produces the barbules.

If, however, the starving period is prolonged beyond the third day *a marked reduction of linear growth occurs* (Table I. gives exact figures for the growth of the rectrices of two starved, and two control birds). This means that *under poor nutritive conditions the formation of barbule-forming cells is first checked, and only under still more unfavorable food conditions will the growth of barb-forming cells be impaired.* This is a fact of the highest importance for an understanding of the origin of fault-bars, and also for a proper conception of the basis for the rhythms of pigmentation (formation of the fundamental bars). The proof of this is furnished by both microscopic and macroscopic study. We have already referred to Fig. 25 of Pl. XV., which shows the halted growth and division of barbule-forming cells in the region of the fault-bars.

In connection with these facts concerning the region and rate

[1] This bird was found to be in bad condition and died soon after this series of observations was concluded.

of growth we may consider one or two other related points. In the rectrices of doves the distance separating the point where the barbule cells arise, and a point at which they are practically completely cornified, is less than a centimeter. In his paper on the Development of Color, Strong's drawings show practically the same for *Sterna*, though he does not call attention to the fact. The measurements of the rate of growth (6 mm. per day) in the rectrices of the dove, when considered in relation to the distances separating the *origin* and *cornification* of a barbule cell, show that the *entire formation and differentiation of these cells occur in the short space of 24 to 36 hours.*

Since, moreover, 6 mm. of linear growth is accomplished in the 24 hours, 1 mm. would be grown in 4 hours. This would mean, then, that where a defective area (absent barbules) is 1 mm. in width that this area was produced in approximately 4 hours. This cannot be otherwise since the barbs — on which the barbules are borne — have been shown to grow steadily on during fault-bar producing conditions; certainly until the third or fourth day of starvation. *A defective area 1 mm. wide in the rectrices of doves indicates, quite certainly, very low nutritive conditions during a period of about four hours.*

The Nutrition of the Feather.

General on Feather Nutrition. — Hypotheses innumerable, concerning the nutrition of the feather, bridge the gap between the very queer conceptions of Dutrochet, Cuvier and Chadbourne. Dutrochet[1] asserted that the "blood-vessels are strangers to the phenomena of feather nutrition"; that a little liquid merely filters upward through the papilla. Cuvier[2] taught that the feather is laid down in a mould or matrix, the freshly added particle accomodatingly taking its place on the outside of parts already formed. Chadbourne[3] has informed us, however, that not only does the body establish blood and "vital" relations with the

[1] Dutrochet, R. J. H., "De la structure et la régénération des Plumes," *Journ. de Physique*, LXXXVIII., 1819.

[2] Cuvier, F., "Observations sur la structure et le development des Plumes," *Mem. de Muséum*, XIII., 1825.

[3] Chadbourne, A. P., "The Spring Plumage of the Bobolink with Remarks on 'Color Change' and 'Moulting,'" *The Auk*, Vol. XIV., No. 2, 1897.

feather-germ, but that the latter sustains and enjoys these same relations long after its maturity.

In discussing feather nutrition attention may be called to certain general nutritive relations which the feather bears to the surrounding parts. It has been pointed out by Poulton[1] that the feather follicle itself is merely a mechanism whereby "a better nutrition and support" of the feather is attained. The extreme vascularity of the papilla is another condition favoring a high nutrition. Further, the pulp cavity is widest and the epidermal parts thinnest at the region of most active growth (Pl. XII., Fig. 1).

Structural Relations Between the Feather-elements and the Blood. — A word is necessary here concerning the more intimate relations between the blood and the growing feather-elements. From Pl. XII., Figs. 4–5, much of these relations can be seen at a glance. It will be noted that (1) the capillaries are extremely numerous along the outer edge of the pulp; (2) of the epidermal structures the thin cylinder-cell layer lies nearest the capillaries; (3) the barb-forming region is narrowly separated from the capillaries by the cylinder-cell layer; (4) the barbule-forming region is still further removed from the capillaries; (5) lymph spaces extend presumably from the pulp to the outer sheath; (6) the large pigment cells occupy positions between the cylinder-cell layer and some barb-forming cells on the one hand and the barbule forming cells on the other.

It is obvious that the cylinder-cell layer occupies the favored position as regards all exchanges with the blood. It is an observed fact that its component cells continue to divide longer, *i. e.*, at a higher level in the feather, than any other part of the germ. Further, the barb-forming region is in a more favored position than the barbule-forming portion, the former being closer to the capillaries, and is also able to profit by adding to itself some of the newly formed cells of the cylinder-cell layer. That the barbules actually suffer more than the barbs under reduced feeding, etc., is proved by the structure of every fault-bar, and by reduced cell-division and growth of this region, as demonstrated by sections. This is also shown by the seven-day starving experiment already cited.

[1] Poulton, E. B., *The Quart. Jour. Micr. Sci.*, Vol. XXXVI., 1894.

From the above described relations it is plain that in case of a poor blood supply to the germ, the distant (more external) barbules would be first to suffer, since all their nutriment must filter through the cylinder-cell layer and around the barbs in order to reach them. The cylinder cells by their shape (usually spindle-shaped) are especially adapted to take up a maximum amount of food, so that parts peripheral to these will certainly suffer first.

It now becomes evident that the low blood pressure produced by amyl nitrite, and the low pressures (discussed below) occcurring normally, must exercise their chief effects on the distant barbule cells; a flow of lymph *from* the latter occurring as soon as the capillary tension is reduced. It is also plain that such a movement of the lymph must act upon these rapidly dividing cells in the same way as would an actual reduction of food with the blood pressure remaining constant. It is upon extremely actively dividing cells, which are removed by several cell diameters from the capillaries, that changes in the vascular pressure have an opportunity to exert an influence. Could more favorable conditions be imagined?

This daily rhythm of accelerated and depressed mitosis recalls similar cases in plants. It is well known that the cells of the root-tips of the Windsor bean (*Vicia faba*) show the greatest mitotic activity at noon — 11:00 to 1:00 daily. *Spirogyra* shows most rapid division at 1:00 A. M. and this period of maximum activity may here be delayed for several hours by cooling. In the plants it is difficult to determine the actual cause of the rhythm. In the feather cells, however, it is certain that the period of fewest mitoses is the period at which least food is available.

Blood Pressure Rhythms in Birds.

It must be said that a *daily blood pressure curve* for birds has not yet been produced. Probably no one, however, will doubt that there is a rhythm of high and low pressures in birds. Probably too, physiologists generally would expect the lowest point of the curve to correspond to the night — as in mammals. In fact, what we know of the factors upon which blood pressure depends in mammals, and what we know of the structures, habits and physiology of birds, would compel us to ascribe to the latter

a relative high pressure during the day and a low pressure at night. I give these *a priori* reasons for believing that a low blood pressure exists in birds at night because some may not be disposed to accept this as proved on my evidence alone.

To me it seems that the result of the amyl nitrite experiment proves much more than that a lowering of the blood pressure will produce fault-bars. *It is significant that the fault-bars produced by the amyl nitrite were superposed upon the defective lines* (and *not* on the fundamental bar between them) *belonging to the particular days of the experiment* (as was determined by subsequent examination). From this we can say with absolute certainty that *the fault-bars are normally laid down at night.* Furthermore, the smaller, "normal" low pressures must be accredited with the *same action* as these observed *extremely low* pressures and are, therefore, to be associated with the less obvious defective lines which are produced daily; they exert their action in the same direction, but to a smaller extent.

In this connection we may quote Tigerstedt's[1] statement concerning the blood pressures of poorly nourished animals. This author states that "schlecht ernährte Thiere haben einen niedrigern Blutdruck als kraftige Individuen derselben Art."

Finally, we may state the following inter-related facts, which go very far toward proving that a low blood pressure normally occurs at night in birds:[2] (1) Diminished feeding of birds produces emphasized fault-bars. (2) Artificially reduced (amyl nitrite) blood pressures produce equivalent defects. (3) The fault-bars are universal in feathers. (4) The fault-bars are produced at night. (5) The lowest daily temperature in birds occurs from 1:00 A. M. to 5:00 A. M. (6) Other physiological conditions of the bird seem to be favorable at night for the production of low blood pressures. (7) A lowering of the pressure would reduce the food-supply and have a tendency to produce defects.

Material for a direct demonstration of the daily blood pressure curve has unfortunately not been available. That its minimum

[1] Tigerstedt, R., "Lehrbuch der Physiologie des Kreislaufs," Leipsic, 1893.

[2] Simpson and Galbraith whose work is cited below find that the temperature curve of the owl (nocturnal) is reversed. If this is true the blood pressure curve of this bird may also be reversed, and the fault-bars (and light fundamental bars) produced probably in the afternoon.

occurs at night is shown, however, by the facts stated above. Duerden has been urged to obtain this curve from the ostrich. He writes me, however, that in his opinion the extremely nervous ostrich is not well adapted for such experiments.

Temperature Rhythms in Birds.

The daily temperature curve of birds was first obtained by Corin and van Beneden.[1] They found that the lowest temperature in pigeons occurs at 4:00 A. M. and the maximum at about 4:00 P. M. Their published curve is not altogether convincing because of the single species and few specimens examined, and because the temperatures at the close of their 24 hours' observation were always lower than at the beginning. This undoubtedly means that the first and highest part of the curve is too high. It was doubtless produced by the fright and struggles of the bird upon the first few insertions of the thermometer into the cloaca.

The only other observations on the temperature curve of birds which I have been able to find are by Simpson and Galbraith[2] who have recently obtained curves similar in most respects to those of Corin and van Beneden, and to those here reported by the writer. The work of these investigators showed, moreover, that in at least one nocturnal bird — the owl — there is a reversal of the diurnal temperature curve.

Corin and van Beneden reported that there is a fall in temperature from 8:00 A. M. till mid-day. Simpson and Galbraith did not find the same. This and other discrepancies in the results reported, together with the fact that only a few birds had been examined, led the writer to repeat the temperature observations on 16 birds. I, too, fail to find a fall in temperature between 8:00 A. M. and noon; only four showed a fall, nine a rise, and three no change in temperature between these hours. It is quite probable that by taking the temperature of the birds during every hour of the twenty-four, as it was done by Corin and van Bene-

[1] Corin, G., and van Beneden, A., "La Regulation de la température chez les pigeons," *Arch. de Biologie*, Vol. VII., pp. 265–276, 1887.

[2] Simpson, S., and Galbraith, G. G., "An Investigation into the Diurnal Variation of the Body Temperature of Nocturnal and Other Birds, and a Few Mammals," *Jour. of Physiol.*, Vol. XXXIII., December, 1905.

den, the normal condition of the birds was disturbed, and not being able to rest sufficiently at night they rested later — from 8:00 to 12:00 — and therefore showed a falling temperature during these hours.

The combined temperature curve for 16 birds, *i. e.*, 6 ducks, 5 ring doves and 5 chicks, is here reproduced (Fig. 5). The birds were under continuous observation for 48 hours. The curve expressses the temperature during the last 24 hours. The readings taken during the first 12 hours were found to be too high and unreliable; these were thrown out and the error of Corin and van Beneden avoided. This information was gathered with a view to obtaining additional data concerning the probable time of the lowest blood pressure in the bird. If the diurnal temperature

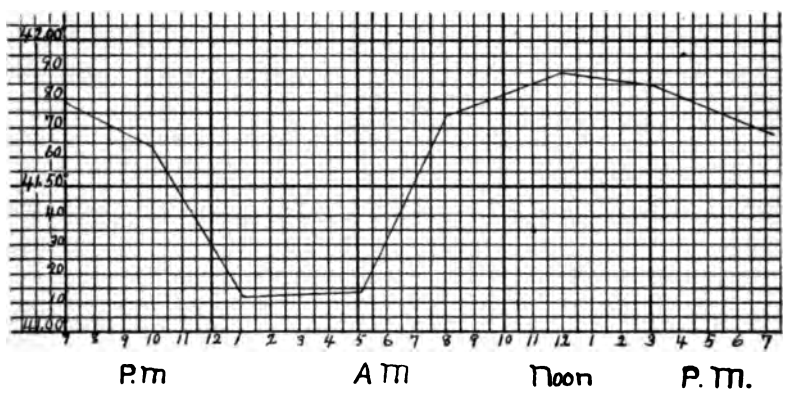

FIG. 5. Combined diurnal temperature curves of 16 birds.

and blood-pressure curves in birds are similar curves — as they are known to be in mammals — the temperature curve shown here indicates that the lowest blood pressure in birds occurs from 1:00 to 5:00 A. M. If the fall in the blood pressure is as sharp and of such short duration (3 to 6 hours) as is the very low temperature, it is easy to understand how it is that only a faint, narrow *line* is usually left to record its action.

Since the daily variation in temperature in birds is shown to be considerable — the above figures showing a lower temperature of about 0.7° C. for four hours of the day than for another twelve hours, — and since the fault-bars are produced during this low temperature, it may be asked whether the reduced temperature is caus-

sally related to the production of the fault-bars, and of the reduced pigmentation (light fundamental bars) soon to be considered. This is a possibility which should be considered in view of the fact that a reduction of temperature of 0.7° C. will, according to the rule of van't Hoff, reduce the speed of chemical reactions by one fifteenth. Furthermore, it has lately been stated by Tower[1] that in insects (where most experimental work on coloration has been done) "the most potent factors in the modification of color are temperature and moisture" (p. 214). I do not believe, however, that temperature is an important factor in the cases we are now considering. The various parts of the *growing region* of the feather undoubtedly have the same temperature and yet they grow unequally; this unequal growth of parts such as pigment, barbs and barbules under fault-bar producing conditions is really our whole problem, and has been proved to be caused by a factor — a reduced nutrition — which affects these parts to a different extent.

Relation of Pigment to Fault-Bars and Nutrition.

The position of the pigment cells between the cylinder-cell layer and the barbule-forming cells has already been pointed out. It will be noted, too, that their processes reach the barbule cells and are, in fact, rather closely applied to them at the time the latter secure their pigment. In just the same way that a lack of nutrition checks the production of barbule-forming cells, it reduces the amount of pigment formed and taken up by the barbule cells.

It seems very certain that this last statement is true, for a macroscopic examination of the completed feather shows a deficiency of color in this region; and some parts of the cross-sections of such areas of adult feathers show plainly upon microscopic examination that less pigment was produced there. It is, however, very difficult to show by microscopic means that in the living, growing feather-germs less pigment is being produced, because of the fact that the pigment cell processes are never able to even approximately empty themselves, and always appear

[1] Tower, W. L., "Evolution in Chrysomelid Beetles of the Genus *Leptinotarsa*," Carnegie Institution, 1906.

black. Quantitative estimates by this method are therefore not easy. Pls. XII., Fig. 4, and XV., Fig. 26, and text Fig. 2 show, very clearly that in these two types of fault-bar there was practically a suspension of pigment production during the fault-bar producing period.

In Pl. XIII., Fig. 14, is shown a feather from a Japanese turtle dove. Here a continued reduction of the pigmentation of nearly all of the primaries followed a period of malnutrition. This period is indicated in the primaries by a narrowing of the feather-vane at one point and by a few fault-bars. The rectrices of the same bird, recorded this starving period by means of a few very obvious defects. (The actual starving of this bird was neither intended nor observed, but this interpretation seems unquestionable). In other cases it has been observed that a very strong fault-bar sharply separates a pigmented part of a feather from an unpigmented part. Pl. XIII., Fig. 10, shows such feathers from a pigeon. The part of the feather proximal to the defects is unpigmented, although it normally bears pigment.

The observation that fault-bar producing conditions may occasionally weaken or apparently permanently destroy the pigment-producing power of the feather would seem to be of much importance; but the mechanism of this action is wholly unknown. The fact that these fault-bars are areas provided with a reduced amount of pigment, and the further fact that nutrition stands in a causal relation with the fault-bars — and the reduced production of pigment — suggests however some interesting relations of the melanin pigment and the food. Indeed one cannot review the results of these studies without finding some evidence that *melanin arises not from hæmaglobin but from the proteids of the foods (serum) or of the cell.* Recent work in physiological chemistry has furnished much evidence in favor of this view. It would seem that in the birds the production of feather pigment stands in a rather immediate relation to the foods, and even to fluctuations in the food supply.

In regard to the behavior of *lipochrome* pigments, it may be said that, though a thorough study has not been made, they seem to take up their positions without regard to the fault-bars and fundamental bars. Where the word "pigment" is unqualified in this paper, *melanin* is meant.

The Meaning and Cause of Fault-Bars and Fundamental Bars.

The facts bearing on fault-bars and, indeed, on fundamental bars, have already been stated. It remains only to point out their inter-relation and significance.

It has been shown that there exists in certain elements of the rapidly growing feather-germ a rhythm of growth which is dependent upon the nutrition. Those parts of the feather which are grown under the poorest nutritive conditions show defects — *fault-bars* of all grades of imperfection. Those regions of the feather — normally the larger part — which are produced while growth and cell-division are in full swing, form the *fundamental bars.*

In pigmented feathers the development of pigment is modified at night along with the other elements and there results a structurally weakened and less pigmented area. This region we have thus far spoken of as a fault-bar; since, however, this same area has been found in some cases to lose considerable pigment without having lost any barbules, we speak of it also as a *light fundamental bar.* On the other hand, pigment develops uninterruptedly during those hours of the day when growth is most rapid, and the well-pigmented portion of the feather then laid down forms the *dark fundamental bar.*[1]

Fault-bars and fundamental bars are universal in feathers (in white feathers there is, of course, no rhythm of pigmentation), and are direct expressions of the rhythmic nutritive[2] conditions. The poorest food conditions obtain at night. A reduced blood pressure, probably much emphasized in the later hours of the night is to be regarded as a factor (by affecting the nutrition) in

[1] This is a complete confirmation of a view arrived at by Professor Whitman in 1903 (not published until 1907). From observations quite different from these, he had reason to believe that the bars might be "zones of daily growth (light = day; dark = night, or vice versa)."

[2] The words "food" and "nutrition" are used in a general sense and include *oxygen.* It is not improbable that the slower growth and cell division and the diminution of the production of melanin pigment under fault-bar producing conditions are, in part at least, due to a reduced oxygen supply. In all my experiments, and in every lowering of the blood pressure, the oxygen supply of the tissues is diminished. The probability here stated grows in importance when it is remembered that free oxygen plays an important rôle in the germination of seeds, the segmentation of ova (mitotic activities), and probably also in the oxidation (Samuely) of tyrosin (Gessard, v. Fürth and Schneider) to form melanin.

the production of all fault-bars and of the light fundamental bars; while the better normal nutrition of the day and of the first part of the night is associated with the production of the dark fundamental bars.

The large light and dark transverse bars with which we have all long been familiar in Plymouth Rock fowls, in hawks, in jays, etc., are of course not the light and dark fundamental bars with which this work deals, and are not each the growth of a day or night; it is perfectly evident that in them a single broad black or white bar may include the growth of two, three or more days. But even these broad bands of white and black may later be found to bear secondary or derivable relations to the fundamental bars.

The alternating light and dark fundamental bars are only rarely seen in their fullest development, *i. e.*, as well defined alternating bands of lighter and darker color. Experience would indicate that they are found to best advantage in pale feathers rather than in those with a superabundance of pigment. The separation of the feather into the faint defective lines, and the broader well-grown areas is, however, easily found in all feathers. The light and dark fundamental bars are shown in Pl. XIII., Figs. 15–16.

Discussion.

It is then through such a mechanism as has been described in the foregoing pages that the melanin pigment of feathers comes to be laid down in alternating light and dark transverse bars.

In concluding the presentation of this subject, I shall attempt no extended discussion of the relation of this to similar work, nor of its relation to general biological problems. A few statements of this import, touching upon that part of this work which deals with color-formation may, however, not be too wide of the mark.

It can be said that thus far those who have essayed to carry the puzzling facts and phenomena of animal coloration from the dark fields of *heredity* into the proved and clarified domains of *physiology* have met with only partial success at best. The efforts of Graf and of Loeb in this direction were mentioned in my introductory statement as among the most successful.

Graf's contribution is that in leeches certain tissues are found to offer greater resistance than others to the migrating "excre-

tophores" which contain a pigment. At the points of resistance chiefly the muscles of the body wall — the pigment is piled up and contributes strikingly to the color pattern.

The earlier work of Eisig[1] on the coloration of the Capitellidæ presents certain analogies to the work just mentioned. This investigator states that in these forms certain color areas arise through the transformation of blood-disc clusters which lie between the cuticle and hypodermis; the transformation being due to stagnation of the blood flow after invasion by excretory (pigment) particles. Each of the two works just cited undoubtedly throws much light on the coloration of the forms studied; but it may also be remarked that the *origin* of the pigment in both cases is still somewhat in question, and that the results do not seem to be of wide application. In fact, it is now evident that no theory which considers pigments as waste products, tossed about by the circulation until they find some sort of excretion, can be of general application.

Certain pigmentary colors in the color-patterns of several orders of insects have been pointed out from time to time by various workers as being correlated with certain structures — spiracular openings, venation, attachments of muscles in the body wall, positions of other internal organs, etc. In all these cases, however, little more than "correlation" has been accomplished. We have yet, I believe, to obtain anything approaching a complete and unequivocal explanation of any of these associations.

The facts observed by Loeb on the chromatophores in the yolk-sac and embryos of *Fundulus* are perhaps the most helpful of the few similar studies thus far made on vertebrates. The observation of an actual and definite *migration of entire chromatophores* (of two types) into a definite color pattern, and this under some (?) influence exerted by the *circulating blood* forms two important steps toward a physiological explanation of the coloration in question. It should not be overlooked, however, that the final step would involve a much better knowledge of the nature of the influence which the blood brings to bear on the chromatophores; and further it may be said that in the explanation of the color-patterns of animals it seems at present that this mechanism, too, has a rather restricted application.

[1] Eisig, H., "Die Capitelliden," Naples, Monograph, 1887.

Zenneck[1] has shown that in the case of the ringed-snake (*Trophidonotus natrix*) the three longitudinal rows of spots in the adult correspond to the positions of three subcutaneous blood-vessels of the embryo. This work in some respects parallels that of Loeb, but with the important difference that Zenneck finds the pigment aggregating about degenerating vessels only, while Loeb found that the circulation of the blood in the living vessel was essential to the process. That this interesting observation, too, merely throws light on a detail — not a fundamental — of animal coloration, seems quite certain.

A paper by List[2] describes as universal for vertebrates the relations between pigment and blood-vessels which were later described by Loeb for *Fundulus*. His rather theoretical work is based on the erroneous view that the pigments of vertebrate color-patterns are carried into the integument by the leucocytes of the blood. That his conclusions are wrong — particularly as applied to birds — is proved by the results set forth in this paper, and by much other evidence as well.

Following this very brief statement and criticism of previous work, we may perhaps be pardoned a concluding word concerning the scope and limitations of the present contribution. The limitations of the work here presented are, indeed, as obvious as they are real, but to hold them in bolder relief for a moment may prove of some slight service to those — if such there be — whose eyes are ill-accustomed to the lights and shadows which play upon this particular section of the borderland of heredity and physiology.

1. The origin and distribution of melanin pigment only has been considered. This includes nearly all the black, brown, and reddish-brown pigments of animals. It is clearly the prevailing integumentary pigment of mammals, birds and many other groups.

2. The birds only have been used as a basis of study.

3. Very few of the actual groupings of melanin pigment in the birds receive an *immediate* explanation through the processes described. It seems very probable, however, that many com-

[1] Zenneck, J., "Die Anlage der Zeichnung und deren physiologische Ursache bei Ringelnatter-embryonen," *Zeitschr. wiss. Zoöl.*, LVIII., 1894.

[2] List, H. J., "Ueber die Herkunft des Pigmentes in der Oberhaut," *Biolog. Centralbl.*, X., 1891.

plex patterns will be found to have been built directly out of the fundamental bars.

On the other hand, the following definite things have been accomplished in the reduction of the "inherited" color-phenomena of birds, to an intelligible, physiological basis.

1. All the feathers of all birds possess — usually in addition to other coloration — regular segmental color-characters which represent (roughly speaking) days and nights of growth.

2. The darker portions are produced by day and the first part of the night, the lighter portions chiefly at night between 1:00 and 5:00 A. M.

3. A low blood pressure at this latter period produces a reduced nutrition.

4. This reduced nutrition causes a slower rate of growth of most — but not all — of the feather elements. The pigment is one of the elements which suffers a reduction.

5. The pigment (and certain other parts) is reduced in its rate of production relative to growth in certain other parts, because of the less favorable relations which the pigment producing cells bear to the nutriment carried by the blood.

The writer believes that these findings give a clearer and more penetrating view of the genesis of a color character than has heretofore been obtained. In no previous case has the attempted analysis included *reasons for quantitative variations in pigment production.* In fact, the whole matter of the origin of pigment — as distinct from the problems of its distribution or placement — has in all these cases been either left untouched, or only waste products acting as pigments have been considered; or again, gratuitous assumptions have been made of its origin from hæmoglobin.

Probably, however, the chief value of what is here presented lies not in the moiety it has taken from the province of heredity and added to that of physiology, but in the *absolute starting point which it supplies for evolutionary studies of the color-characters of birds.* Whitman[1] has already pointed out that the *fundamental bars, whose significance is here made known, are to be re-*

[1] Whitman, C. O., "The Origin of Species," *Bull. of Wis. Nat. Hist. Soc.*, January, 1907.

garded as the primitive avian color-markings. He further states that "from these fundamental feather-bars or their secondary derivatives a multitude of specific characters have been evolved by gradual modification."

SUMMARY.

1. Fault-bars, or feather defects presenting five rather distinct types, have been found and described.

2. The fault-bars occur "normally" in all bird groups, in all plumages, in all feather tracts, and in all individual feathers.

3. Fault-bars can be readily produced experimentally by reduced feeding; by the feeding of the fat stain Sudan III., which seems to "tie up" certain foods; by very strong mechanical crumpling sufficient to break the tissues and blood vessels; and by lowering the blood pressure with amyl nitrite.

4. Fault-bars are produced only by such agencies as bring about poorer nutritive conditions in the feather-germs.

5. Homologues of the fault-bars are to be found in the several epidermal growths of animals which reflect better and worse nutritive conditions. Other tissues than the epidermal may be found to show structural effects of rhythms of growth.

6. The region of growth in the feather is very restricted, being narrower for the barbule — less than 1.5 mm. in very large feather-germs — than for the barb-producing region.

7. The rate of growth in the feather, as compared with growth elsewhere in the organism is extremely rapid, and varies within wide limits. Up to a certain point the rate bears a rather definite ratio to the ultimate length of the feather, being fastest in those feathers which become longest.

8. Under "starving" conditions, the rate of linear feather-growth is not affected until the third or fourth day; after this the rate falls rapidly (doves).

9. The structural relations between the various feather elements and the blood are such that not all the parts are equally favorably situated to obtain nutriment from the blood; the shaft, barbs, pigment, barbules, inner and outer sheaths occupying advantageous positions in the order named.

10. Previous results showing that the lowest (daily) temperature of birds occurs in the early hours of the morning (1:00 to 5:00 A. M.) have been confirmed.

11. A daily blood pressure rhythm with a minimum pressure occurring doubtless between 1:00 A. M. and 5:00 A. M. is present in birds.

12. The reduced nutrition brought about daily by this minimum blood pressure; the disadvantageous position, in relation to the blood, of the pigment and barbule elements of the feather; together with the very rapid rate at which feathers grow, furnish the complex of conditions which bring unfailingly into existence a fault-bar, and to a more or less appreciable extent a light fundamental bar, at perfectly regular intervals in the entire length of every feather formation.

13. The melanin pigment of the feathers of birds shows, under favorable conditions, quantitative variations of the pigment produced in response to changes in the available food supply. This is an additional evidence that this pigment is not a derivative of hæmoglobin, but of the serum or cell proteids.

14. The light and dark fundamental bars are universal in feathers (Whitman) and have been shown by the writer to represent different conditions and periods of growth; the dark fundamental bars (of pigmented feathers) being expressions of growth and pigment production under the most favorable conditions; these conditions obtain during the day and the first part of the night; on the other hand, the light fundamental bar is brought into existence, as stated above, by fault-bar producing conditions, *i. e.*, by the low nutritive conditions which obtain during the later hours of the night.

15. These results furnish a description in the terms of physiology, of the mechanism of the "inheritance" of certain fundamental color-characters of all birds.

16. The fundamental bars furnish the starting point for all evolutionary studies on the color-characters of birds.

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ABBREVIATIONS.

b. fault-bar.
bb. barb.
ble. barbule.
c. region of reduced pigmentation.
cap. capillaries.
if. inferior umbilicus.
i sh. inner sheath.
lb. longitudinal fault-bar.
m.b. basal membrane.
m. modified region.
n. nucleus.
p. pigment cell.
pc. process of pigment cell.
pl. pulp.
rh. rhachis or shaft.
tu. outer sheath or tunica.

Explanation of Plate XII.

Fig. 1. Diagrammatic representation of rectrix of dove in longitudinal section. Barbule cells are formed between *x* and *y*; barb cells in the region of *z*; cornification complete at *w*; Fig. 5, Pl. XII., from region *v*. × 14.

Fig. 2. Semi-diagrammatic cross-section of a feather-germ. × 50.

Fig. 3. Longitudinal section of feather-germ of *Cardinalis;* constrictions *a* invading pulp. Drawn with aid of camera lucida. × 20.

Fig. 4. Modified region of a feather from a "starved chick." Effects of lack of food are seen in the loss of barbules in the fault-bar region *a*; in a very peculiar massing and cornification of barbs at *m*; and in an almost total absence of pigment in the shaft at *c*. × 8.

Fig. 5. Camera lucida drawing of a segment of the cross-section of the rectrix of a dove, taken at the level *v* in Fig. 1. No attempt was made to indicate the cellular structures of the pulp; the red blood corpuscles within the capillaries also omitted. Only such capillaries were indicated as contained red blood cells and thus made their nature certain. Undoubtedly still others were present. The line across the four ridges indicates the plane of section of Fig. 25, Pl. XV. × 360.

EXPLANATION OF PLATE XIII.

All figures are direct prints on solio paper, therefore white shows as black, and *vice versa*.

FIG. 6. From a young dove which fed normally during the first week after hatching, poorly fed during the second week, and fed still less during the following ten days.

FIG. 7. Tail covert of an underfed dove. "Abrasions" *a* occurring at the fault-bars. Those at *d* produced artificially by pulling on distal end of barbs.

FIG. 8. Body covert (from nape of a Sudan-fed chick) with wavy band crossing it; before this region expanded it existed as a constriction (fault-bar type 4) of the feather-germ.

FIG. 9. Series of the same modifications in body covert of *Cardinalis*.

FIG. 10. From region of crop of pigeon. A fault-bar occurs at *b* which sharply bounds a peripheral pigmented and a proximal unpigmented portion.

FIGS. 11–12. From tail of English sparrow. No. 11 has an incompletely differentiated, unexpanded portion *b* at a point where the feather which grew beside it (Fig. 12) shows a typical fault-bar.

FIG. 13. Primary of chick (juvenal plumage) showing fault-bars produced by feeding Sudan III.

FIG. 14. Primary of Japanese turtle dove. A fault-bar at *b* separates a more pigmented distal from a less pigmented proximal part. The entire feather is narrowed from *b* to *a* showing that poor nutritive conditions prevailed throughout this period. This region seems, by the method of photography here employed, to be more heavily pigmented than other parts; this is due not to actual pigmentation but to an opacity caused by the extreme cornification and lack of separation of the feather-elements.

FIG. 15. Showing light and dark fundamental bars in body covert of a Japanese turtle dove.

FIG. 16. Fundamental bars in wing covert of pigeon (these rather faint bars and those of Fig. 15 are here practically lost. The method of direct printing here employed is not equally good for the fault-bars and fundamental bars).

FIGS. 17–19. No. 17 — primary, No. 18 — secondary covert, and No. 19 a body covert from crop region of a chick. The difference in distance between successive fault-bars is an index of the rate of growth and bears a definite relation to the ultimate length of the feather (the fault-bars in Fig. 19 are practically lost in the reproduction), though they were very plain in the specimen.

FIG. 20. Crumpled primary of right wing of dove showing fault-bars at *d*.

FIG. 21. Control of above; the corresponding primary of the left wing of the same dove.

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EXPLANATION OF PLATE XIV.

FIG. 22. Four rectrices of chick "dosed" with amyl nitrite on two succeeding nights. (Several feathers had their tips cut off at approximately 18 mm. from the skin, so as to be able later to identify the region affected by the low blood pressure produced.) Points marked *b* are the fault-bars produced on the nights of the experiment.

FIG. 23. A fifth rectrix which in addition to the usual fault-bars shows a longitudinal fault-bar for *lb* beginning with the second night of the experiment.

FIG. 24. A rectrix from the control chick. These feathers show no defects.

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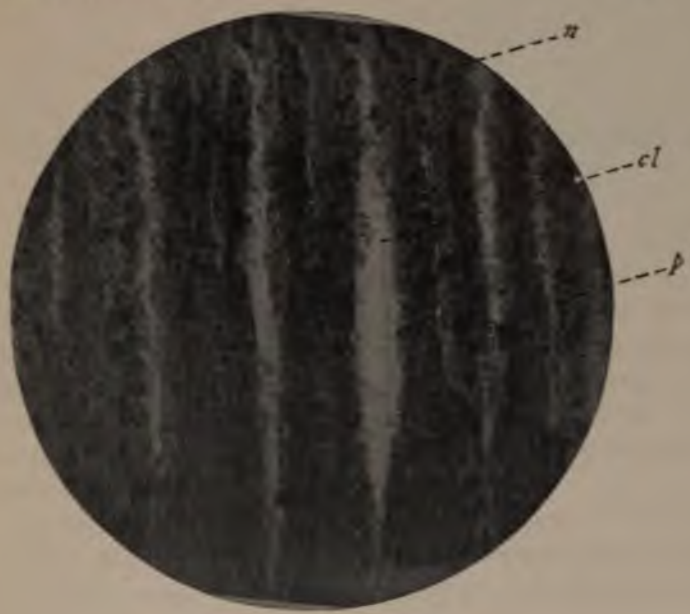
Explanation of Plate XV.

Photomicrographs.

Fig. 25. Longtudinal section of a fault-bar region soon after fault-bar producing conditions were past. This being a tail feather, from the chick which was given amyl nitrite, taken four hours after the chick was removed from the amyl nitrite atmosphere.

This "bar" occurs about 1.5 mm. from the extreme proximal tip of the feather and occupies a position which may be better understood by reference to Fig. 5, Pl. XII. It is a region on the border line of barb, barbule and pigment producing cells. Cell outlines in such a region are usually indistinct; the nuclei *n*, however, have not photographed perfectly, so that an idea of the looser, more irregular arrangement of the cells is only imperfectly given. That an actual reduction of growth occurred here, is, however, beautifully shown by the large clefts *cl* or clear areas of the pulp which have here been left between the several barb-formations (ridges). Killed in Kleinenberg's picro-sulphuric mixture, and stained in hæmatoxylin and eosin. × 300.

Fig. 26. Fault-bar *b* with reduced pigmentation of all elements of the feather, including the shaft *c*, in a rectrix of the ring dove. × 40.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LYCASTIS QUADRATICEPS, AN HERMAPHRODITE NEREID WITH GIGANTIC OVA.

HERBERT P. JOHNSON.

The possession of unusual characters by any species or group of animals always arouses our interest and invites thorough investigation. Among the Annelida Polychæta the genus *Lycastis* in the family Nereidæ has the striking physiological peculiarity that a majority of its known species are found living in fresh water as well as in the sea — a thing of rare occurrence in other families of the Polychæta.

In addition to the above-mentioned anomaly *Lycastis quadraticeps* is an hermaphrodite; and furthermore, instead of producing many small ova it develops only a few of relatively colossal size.

The species was originally described in that comprehensive work of Claudio Gay, "Historia Fisica y Politica de Chile" ('49). The specimens are stated to have been collected at Calbuco, on the Chilean coast, and the brief description and two figures give no information beyond the diagnostic external characters. It was redescribed by de Quatrefages in 1865 (Tome II., p. 500), who, however, added nothing new.

Nearly a half century later it was rediscovered by Plate at Lapateia, Beagle Canal; and by Michaelsen, who found it at Punta Arenas, on the Straits of Magellan, living not only in the sea but in brackish water and even in fresh water; always, however, in places accessible from the sea, so that we may infer that the fresh-water habitat has been recently acquired. These observations by Plate and by Michaelsen have been recorded by Ehlers ('97, '00, and '01), who, however, failed to note the striking sexual idiosyncrasies of the species.

When, in 1902, I was engaged in the preparation of an account of certain fresh-water nereids ('03), Professor Michaelson kindly placed at my disposal a few specimens of *L. quadraticeps*. With two exceptions they proved to be sexually mature and her-

maphrodites, containing nearly ripe sperm-cells and a limited number of ova which are enormous considering the size of the parent. These facts have been already briefly recorded in the above-

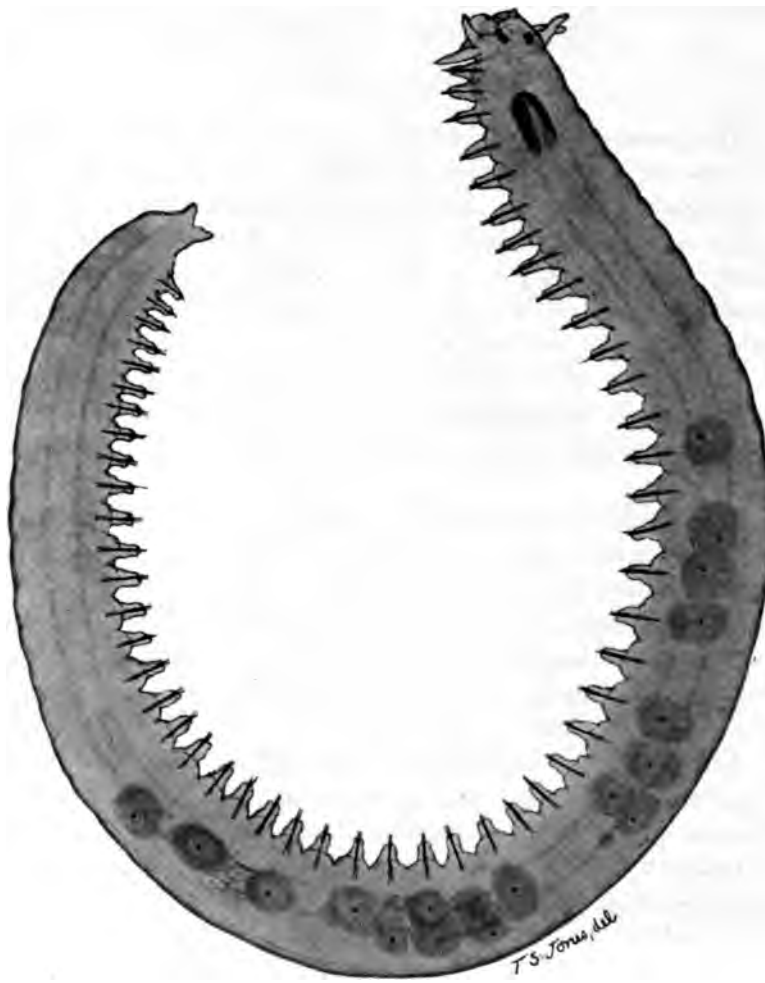

FIG. 1. *Lycastis quadraticeps* Gay. Entire worm, dorso-lateral aspect, unstained, mounted in balsam. The jaws, intestine, and large ova seen through the body-wall. Nucleoli of ova opaque and visible. × 20.

mentioned article (Johnson, '03). Since the species inhabits a region remote from ordinary travel and one cannot reasonably expect ever to see it in the living state, I have thought it worth

while to put on record the few additional facts I have been able to gather from a study of the material at hand.

The species might almost be called minute, considering the generous dimensions of most of its allies. It seldom exceeds 18 mm. in length and one millimeter in transverse diameter, including the parapodia, but not the setæ. While of a general cylindrical form and nearly uniform diameter throughout, there is a fairly marked tapering in the caudal region toward the small rounded pygidium which bears a pair of short, conical anal cirri (Fig. 1).

As in all species of *Lycastis* the feet are uniramous throughout the series, a very small tubercle and an acicula representing the dorsal ramus. The dorsal cirri differ from those of most species of the genus in being diminutive instead of large and foliaceous. Each parapod is armed with a single aristate seta representing the dorsal fascicle, and a ventral fascicle of one aristate seta and three to five falcate ones. The prostomium is characteristically short and broad; the antennæ are minute, and the tentacular cirri all short and stumpy, the stylode tapering rapidly from a short, thick ceratophore. The palpi are large for the size of the worm. The four eyes are black, those on the same side placed close together.

In specimens cleared with oil and mounted entire in balsam the internal structures can be made out in considerable detail (Figs. 1 and 2). No paragnaths have been detected, nor indeed are they known to occur in any species of Lycastis. The jaws are plainly visible and are armed with four or five teeth. The alimentary canal has the usual divisions, the only part deserving especial mention being the glandular stomach, which lies in somites VII. and VIII., or in VIII. and IX., or even extends into X. This "stomach" is thick-walled, exceeding in this respect even the œsophagus, and the lumen is strongly encroached upon by the large rounded protuberances which stud its inner surface. In sections these protuberances are seen to be composed of a syncytial mass containing numerous darkly staining nuclei, but no cell-boundaries have been detected. This is clearly a portion of the lining epithelium of the alimentary canal, but of peculiar and specialized character. In addition to the nuclei there are very many minute bodies, some round and some

elongated, which stain strongly with hæmatoxylin. The muscular layer is no thicker than in other portions of the alimentary canal.

Following the stomach is a constricted portion occupying somite IX. or X., which in turn leads directly to the intestine.

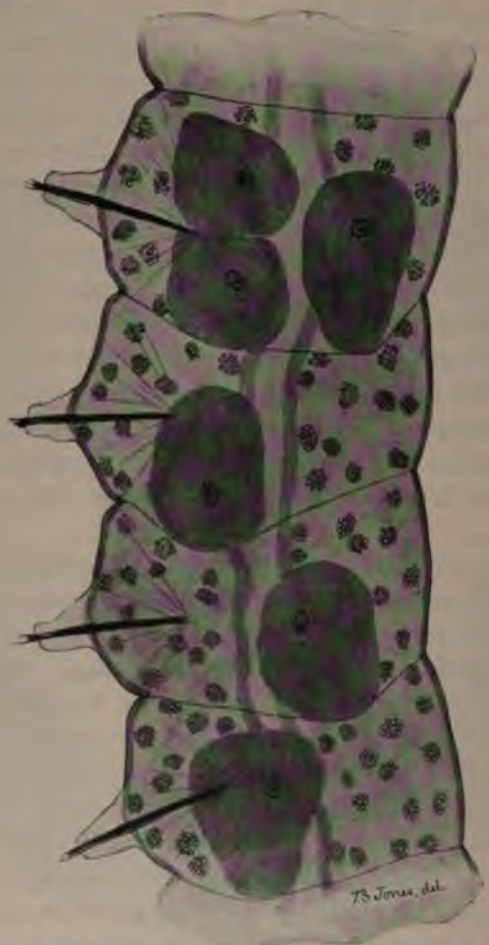

FIG. 2. Portion of another specimen, mounted in same way, more magnified. Bunches of sperm cells, ova, and intestine seen by transparency. × 37·5.

As very commonly in the Polychæta the latter is expanded in every somite and constricted at every septum. In especially well-cleared specimens a beautiful vascular network is seen to cover the peritoneal surface of the intestine.

Where one or more ripe ova occupy a somite they usually lie in contact with the digestive tube and are plainly visible as golden-yellow masses, shining through the body-wall (Figs. 1 and 2).

The sperm-cells, which are invariably in clusters, are only a little less obvious than the eggs (Fig. 2). They are present in the same somites as the ova and in many others where no ova have been found. The relations in this respect are best seen in tabular form.[1]

No. of Specimen.	Length in Millimeters.	No. of Somites.	No. of Somites with Eggs.	No. of Eggs.	No. of Somites with Sperm.	Somites without Eggs.		Somites without Sperm-cells.	
						Ant.	Post.	Ant.	Post.
1	17.3	53	20	23	36	20	9	12	7
2	19.5	56	14	16	38	16	18	12	6
3	14.4	56	18	20	43	12	20	10	7
4	17.5	61	22	32	40	16	19	14	7
5	23.0	58	30	38	42	8	8	10	8
6	13.5	45	9	9	35	12	18	7	3
7[2]	10.0	47	8?	10?	?	13	16	?	?

Inspection of the table shows that there is no fixed relation between the number of the somites and the length of the specimen, which of course has its obvious explanation in the differing degrees of contraction which the worms underwent in fixation. Nor is there a fixed relation between the total number of somites and the number of ovigerous somites, or number of ova; nor between the number of somites and the spermatogenous somites. Thus, between Nos. 5 and 6 there is a disparity of only 13 in the number of somites, but a difference of 21 in the number of their ovigerous, and of 7 in their spermatogenous, somites. The greatest disparity of all, 29, occurs in the total number of ova. Or, stated in percentages, No. 5 has about 22.4 per cent. more somites than No. 6, and 16.66 per cent. more of them produce spermatozoa; but on the other hand, No. 5 has developed over 322 per cent. more ova than No. 6! There is really more difference in size, however, than the difference in the number of the somites would indicate, amounting to 41.3 per cent. in the one dimension

[1] The counts have been made from entire specimens, usually unstained, mounted in balsam. With a species so small such preparations generally permit internal structures to be seen with admirable clearness; yet undoubtedly a few small immature ova were overlooked.

[2] Immature. Most of the ova very small, and sperm-cells not detected with certainty.

of length; hence the difference in productivity is not so great as it seems when we consider the really considerable disparity in size.

Examination of the table also shows how variable is the number of the somites, both anterior and posterior, in which neither ova nor spermatozoa develop. In general, more somites in the anterior region are destitute of sperm-cells than in the posterior, while the reverse obtains with the non-ovigerous somites. In every specimen but one (No. 5) more segments at both ends of the series are without ova than are destitute of spermatozoa; and often twice to three times as many somites contain sperm as eggs. A better balance prevails in specimen 5, which is exceptional, inasmuch as a single ovum has developed as far forward as the 9th somite, one in front of the most anterior sperm-producing one.

Many somites, even within what might be called the ovigerous series, fail to produce ova. Often the gap is only a single somite, in other places it is two, three, or more. The longest intervals have been found between the first ovum and the succeeding one (four somites in No. 2, six in No. 5). It is not improbable that a thorough study by the serial section method would bring to light minute, undeveloped ova in these apparently non-egg-bearing segments; for close inspection of the specimen mounted entire has frequently revealed small ova which were overlooked at first examination. In serial sections such ova have occasionally been found in somites packed with sperm-cells, entirely surrounded by the latter, and each accompanied by a colony of what I regard as nurse-cells (Fig. 4).

The rich golden hue of the mature ova is due to the presence of abundant yolk grains of different sizes (up to 35 microns in diameter) distributed throughout the entire ovum except a peripheral layer of finely-granular protoplasm (Figs. 3 and 5). This, however, does not appear to cover the entire ovum, being absent on the side adjacent to the intestine; but the arrangement suggests strongly that which prevails in the Arthropod egg.

The nucleus, at first very large for the size of the ovum, becomes smaller and smaller proportionally as the size of the ovum increases. (Compare Figs. 3 and 4.) The position is generally

eccentric, and the nuclear membrane is sharply defined; the chromatin, at first scanty (Fig. 4), becomes more abundant in the older eggs (Fig. 3). It has the form of granules rather than threads and sometimes part of it has a peripheral arrangement (Fig. 4).

The nucleolus at all stages of the ovum observed is a dense refractive spherical body, measuring from 10 microns in the

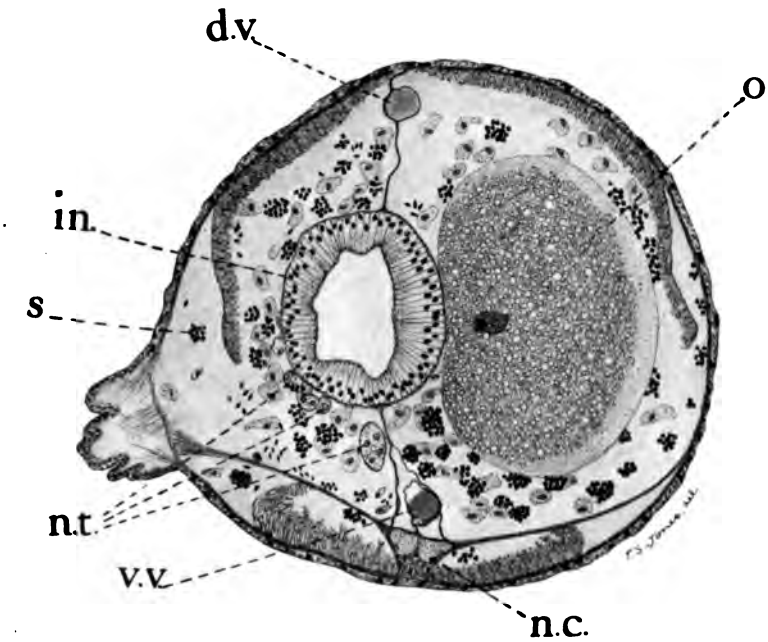

FIG. 3. Transverse section, middle region, stained with hæmalum and eosin. Section passes through an ovum (*o*) and its nucleus. Bunches of sperm cells (*s*) seen distributed throughout the cœlom. The nerve cord (*n.c.*) and transversal muscles extending from it right and left; *d.v.*, *v.v.*, dorsal and ventral blood vessels; *n.t.*, nephric tubule cut across in several places; *in.*, intestine. Amibocytes in cœlom. × 100.

young eggs up to 22 microns in the mature ones. It is eccentric in position, and remarkable for its opacity, being of a brown color by transmitted light and almost white by reflected light. Owing to the strong contrast in color to the ovum it serves as a useful landmark in the enumeration of ova.

The youngest ova and some of the mature ones are accompanied by a group of "nurse-cells." The condition, however, is

not so frequent as one would expect if every egg requires them for its proper development. It is possible there is some way of getting rid of the nurse-cells after the ova have matured, possibly by phagocytosis. This hypothesis is suggested by the fact that the bunch of nurse-cells is most commonly located in a protected place, usually in a sort of pocket between two closely-appressed ova, or between an ovum and the intestine. The aspect of the nurse-cells is closely that of young ova (Fig. 4). The principal reason indeed for not regarding them as ova is the fact that in any group there is never more than one cell that is unmistakably an ovum; it is always clearly distinguishable from the rest by greater size and the presence of yolk granules. If they were all ova one would expect a gradation from the largest to the smallest.

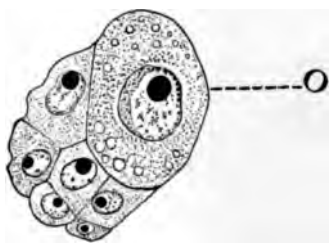

FIG. 4. Young ovum (*o*) with large nucleus, nucleolus, and a few small yolk grains. The smaller cells are considered to be nurse cells. ×412.

What may be the unmodified shape of the ovum, whether spherical or oval, is impossible to determine from eggs still confined in the cœlomic spaces. In every instance owing to pressure upon each other or upon organs of the body the mature ova are more or less deformed. Pressure of the alimentary canal produces a concavity (Figs. 3 and 4); mutual pressure of two or more ova in the same somite also produces a concavity in the more yielding ovum, or else they are flattened against each other. Flattening also occurs with the largest ova where they press against the septa, extending as they do the entire distance from septum to septum. This is sometimes the case even when one or both of the adjacent somites contain no ovum, which gives the impression of unyielding septa or else of very plastic ova. The latter condition is no doubt existent. In one instance an acicula and accompanying fascicle of setæ, owing to contraction of the retractor muscles have made a deep indentation in an ovum, In other places ova are seen to be strongly constricted between two expansions of the intestine; in still other places ova are wrapped, as it were, half-way around the intestine.

Somites with two or three ova never occur near the ends of the

series but always toward the middle. Likewise, the sperm-masses are always most numerous away from the terminal somites of the spermatogenous series.

It would be instructive to compare these ova with those of other species of *Lycastis*. Unfortunately, the material is not at hand to make a general comparison; but it may be not out of place to mention that the eggs of *Lycastis hawaiiensis* are not only much smaller (.15 mm. in diameter) but have a very different nucleus. It is one of the most beautiful examples of a naked nucleus one could expect to find. The nucleoplasm is directly and plainly continuous with the cytoplasm, and the contact of yolk-grains give the nucleus an irregular, almost stellate

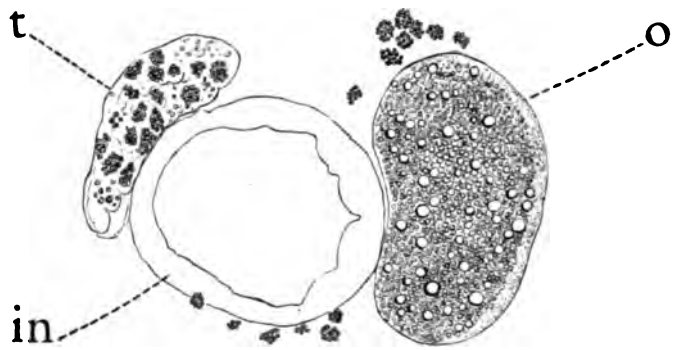

FIG. 5. Section through same specimen, and nearly same region of body, as Fig. 3. "Testis" (*t*) and ovum (*o*) appressed to the intestine (*in*). A few bunches of sperm cells (spermatocytes?). × 112.

form. Generally there is not a single nucleolus of large size, but several small ones. No chromatic network or granules have been detected. There is no peripheral yolkless layer. In the single specimen containing ova they are very numerous, apparently nearly mature, and almost fill the cœlom of each somite. It is thus evident that within the genus *Lycastis* there are species differing widely in the structure of their ova.

None of the sectioned specimens that I have examined have ripe spermatozoa, but the sperm-cells (spermatocytes?) present are apparently all in the same advanced stage of development. Unlike the ova, they occupy every somite from first to last of the series in which they occur, but the clusters of sperm-cells, as

appears in Fig. 3, are not limited to any particular part of the cœlom, but are loose and generally diffuse. Sometimes, however, they are massed in such a way as to give the impression of a definite organ or testis, as shown in Fig. 5. The position with relation to the ovum and intestine here shown is not constant. The spermatocytes in the "testis" are not obviously at a different stage from those free in the cœlom. This male gonad has not been found in all the specimens.

The mode of exit of the genital products offers an interesting problem. It is well-known that in many Polychæta they find their way to the outer world through the nephridia, some of which (as in *Macellicephala violacea*, recently described by Wirén, '07) may be specialized for this function. This evidently has not taken place in *Lycastis quadraticeps*. What little has been made out regarding these organs shows that they are extensive, convoluted tubules with a very narrow lumen, lying just in front of the septum of each somite (Fig. 3, *n.t.*). The lumen does not exceed in diameter one of the sperm-cells. While, so far as appearances go, it is entirely feasible for the spermatozoa to pass out through the nephridia, the same cannot be said with regard to the ova. It is obvious that they can escape only by rupture of the body-wall and consequent destruction of the parent. This is well known to be the only mode of exit in many of the Polychæta. It explains the fewness of ova in immature stages, which must in fact on this hypothesis be regarded as aborted ova, for the death of the parent in ovulation must preclude their completing their development. No immature sperm-cells have been found.

There appears to be no provision against self-fertilization, but it may nevertheless exist in the form of protandry or protogyny.

General Considerations.

Hermaphroditism among the Polychæta is of such rare occurrence that up to 1855 it was supposed to be wholly absent in the group. Even to-day, among the hundreds of known species, very many of which have been studied in their sexual phases, less than a score are known to be hermaphroditic. Although so few, the hermaphrodites are pretty well scattered throughout the entire subclass, as the following list will show. It is probably incomplete.

Macellicephala violacea (Lev.). Incipient; males and females occur. Wirén, '07.

Hesione sicula Delle Chiaje. With paired hermaphrodite organs. Bergmann, '02, '02[a].

Syllis corruscans Haswell. Anterior somites with eggs, posterior with spermatozoa. Haswell, '86; Benham, '96.

Ophriotrocha puerilis Korschelt. Protandric with hermaphrodite organs. Sexes distinguishable. Korschelt, '93; Braem, '93.

Nereis diversicolor Mueller.

Platynereis dumerili Aud. et Milne Edw. Both occasionally hermaphroditic. Caullery et Mesnil, '98.

Lycastis quadraticeps Gay.

? Caobangia billeti Giard. Egg-bearers only observed; male gonads not detected. Giard, '93.

Sabella microphthalmia Verrill. Protogynous paired hermaphrodite organs. Gregory, '05.

Amphiglena armandi Claparède. Ova in first ten abdominal somites; testes in last nineteen. Claparède, '64.

Salmacina dysteri (Huxley). Fissiparous. Male gonads in from three to five anterior abdominal somites; ova in eighth to twentieth. No relation between fissiparity and distribution of gonads. Huxley, '55.

S. ædificatrix Claparède. Ova in anterior abdominal somites, sperm cells in posterior ones. Claparède, '68.

S. incrustans Claparède. Claparède, '68.

Piliolaria militaris Claparède. Claparède, '68.

Spirorbis pagenstecheri Quatrefages. Pagenstecher, '63.

S. borealis. Paired gonads. Ova in first two abdominal somites; male gonads in posterior ones. Schively, '97.

S. lævis Quatrefages. Ova in first two abdominal somites, sperm-cells in all the rest. Claparède, '68.

Among Polychæta in which hermaphroditism has become established we find two distinct conditions. These occur irrespective of the systematic position of the species. In the one case male and female gonads are present in the same somite. Sometimes,

as in *Lycastis quadraticeps*, they have no discoverable relation to each other; in other forms (*Ophriotrocha puerilis*, *Hesione sicula*, *Sabella microphthalmia*), there is a definite hermaphrodite organ (Zwitterdruese). In the other and more exceptional condition the individuality of the somite asserts itself by the production of either male or female gonads. This is best seen in some of the serpulids (notably *Spirorbis*), ova alone being produced in a few of the most anterior abdominal somites, and sperm-cells in a larger number of posterior ones. It is obvious that a combination of this condition with fissiparity, if the plane of division leaves all the male gonads in one zooid and all the female gonads in the other, leads directly to an alternation of generations in which an hermaphroditic parent resolves itself into unisexual offspring. According to Haswell ('86) this actually occurs in *Syllis corruscans*, in which the anterior portion (ordinarily forming the asexual stolon in Syllids) produces ova, and the posterior somites separate as a male worm.

The ova are almost always small or even microscopic among the Polychæta. The egg of *Platynereis dumerili*, measuring .41 mm., has been regarded as one of the largest. Recently, however, Wirén ('07) has found those of Macellicephala to be much larger, and perhaps of maximum size in the entire subclass (.76 by .48 mm.). While the ova of *Lycastis quadraticeps* are not so large absolutely, measuring .43 by .28 mm., relatively to the size of the parent they are far larger.

There are at least two other species of Polychæta which produce very few relatively large ova, — *Nerilla antennata* Schmidt, '48 (*Dujardinia rotifera* of de Quatrefages, '65), and *Amphicorina cursoria* de Quatrefages, '65. Both of these species produce ova not only relatively and absolutely of unusual size, but extremely few in number (de Quatrefages's figures give six in *Amphicorina* and only four in *Nerilla*), and apparently they constitute but a single brood. In these two species therefore, we find the same condition of things as in *Lycastis quadraticeps* — reduction in the number of eggs concurrently with increase in their size — and the process has gone still further than in *Lycastis*.

That this change is not coördinate with complexity of organization or any real advance towards a higher plane of being, but

rather the reverse, is just as evident among the Polychæta as elsewhere in the animal kingdom. As the macroōgenous Cladocera and Aphids occupy but a lowly position in their respective classes, and as the same is true of the auks, guillemots, and apteryx among birds, so we find the macroōgenous Polychætes are all of puny size, comparatively simple organization, and one is hermaphroditic. In what, then, lies the advantage of producing so few ova? Unquestionably, there can be greater storage of food stuff per ovum if the eggs be few, and upon this the well-known law of biogenics, namely, the greater the inheritance of stored-up nutriment from the parent, the greater the chance of survival for the offspring, is based. Whether the young are launched into the world as typical, free-swimming trochophores, as modified trochophores, or in the form of young worms, has not been ascertained in any of these interesting forms. With so large a store of food-yolk a direct or nearly direct development is probable; and the very limited number of eggs presupposes such protection through the early stages that a very high percentage of the young come to maturity.

The occurrence of incipient or occasional hermaphroditism in a few species that are functionally or usually bisexual (*e. g.*, *Macellicephala violacea*, *Nereis diversicolor*, and *Platynereis dumerili*) is of especial interest. In a bisexual form like *Macellicephala* the very beginnings of hermaphroditism may be seen. According to Wirén ('07) undoubted traces of male gonads are never found in female specimens. In those which are functionally male, however, all the gonads contain groups of ova. As in the female, they are each surrounded with a follicular membrane. Free ova also occur in the cœlom, but no mature ones of full size and rounded form. It is impossible to be sure from the few observations whether this species is actually an incipient or an occasional hermaphrodite.

The most carefully-studied hermaphroditic Polychæte is probably *Ophriotrocha puerilis*. In this species, according to Korschelt ('93) it is often difficult to distinguish between males and females; but in the older specimens even in the living state the microscope reveals the ova or spermatozoa in the cœlom. Nevertheless, hermaphroditism is the prevailing condition. Says Korschelt

(p. 274): "Es kann somit kein Zweifel sein, dass bei Ophriotrocha Hermaphroditismus vorkommt und es koenne sogar maennliche und weibliche Geschlechtsprodukte zu gleicher Zeit von ein und derselben Keimdruese gebildet worden, aehnlich wie dies in der Zwitterdruese der Opisthobranchier und Pulmonaten der Fall ist."

There is a strong approach to regional hermaphroditism in Ophriotrocha. The most anterior somites of the genital series produce only male gonads and the female elements become more and more predominant towards the posterior end.

Korschelt distinguishes in Ophriotrocha four sexual phases:

1. Pure females. Male genital products not detected at any stage of development.

2. Pure males. Ova not found.

3. Apparent females. Well-developed female gonads and free ova. Also, male genital products both mature and immature.

4. Apparent males. Well-developed male gonads and multitudes of spermatozoa; ova also present in the gonads.

When both male and female elements are present in the same somite the male are found to be much further developed than the female. These individuals Korschelt believes are functionally males until the female gonads are ripe, whereupon they assume the rôle of females. There thus occurs a protandric hermaphroditism by which self-fertilization is prevented.

Something of the same nature, but with the order of events reversed, was observed by Miss Gregory ('05) in *Sabella microphthalmia*. Specimens examined in April and early part of May were pure females; in August all specimens were either hermaphrodites or females, the latter increasing in proportion as the season advanced. Pure males were not found at any time.

From the few examples that have been sufficiently studied to show the true nature of hermaphroditism in the Polychæta, it is clear that it is highly variable in its manifestations, and hardly of fixed character even in the forms where it appears to be most firmly established. This condition taken in connection with its sporadic distribution leads naturally to the conclusion that it is of comparatively recent origin in the group.

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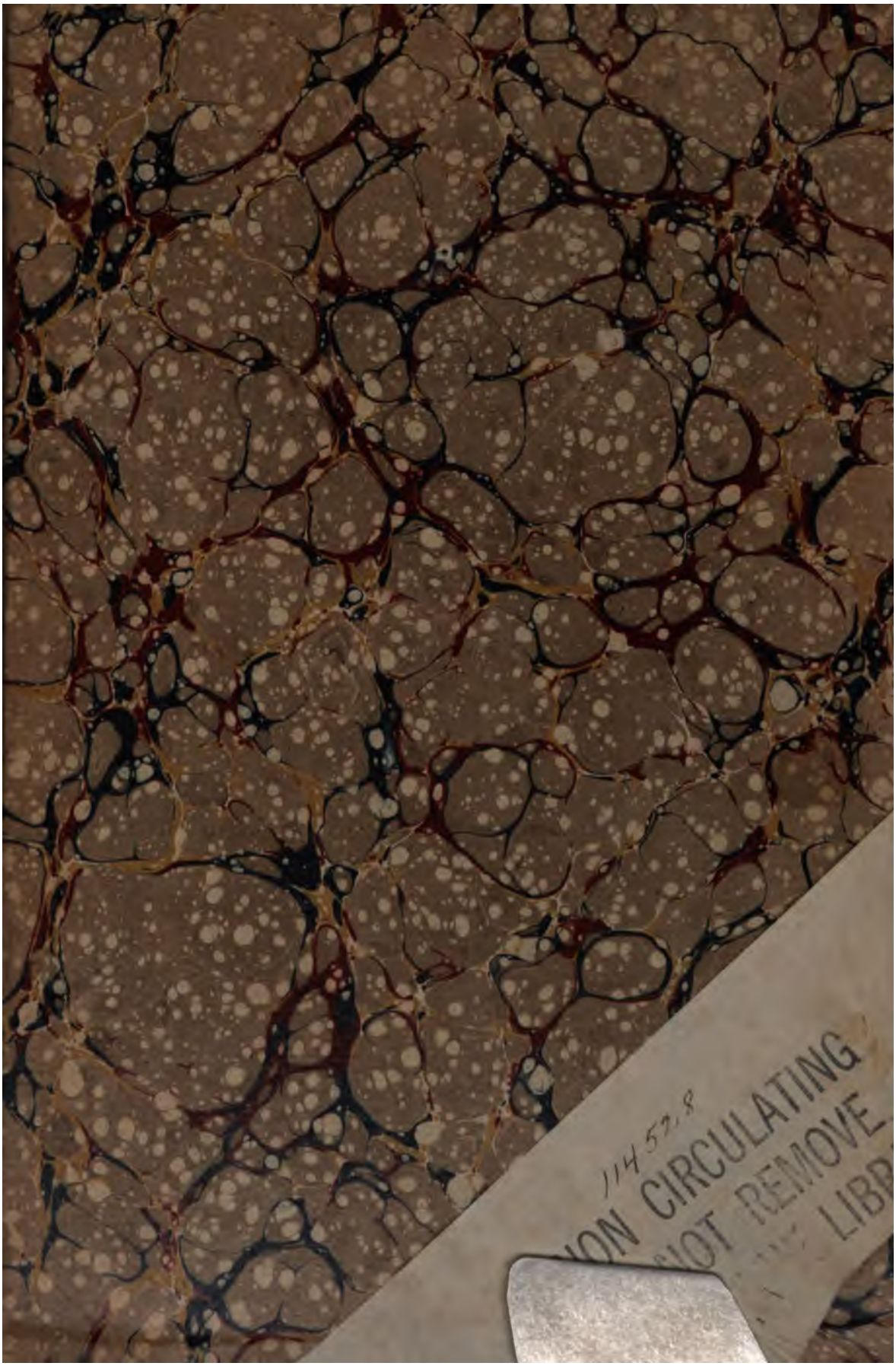
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